Contributions to the Cytology of the Bacteria.

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With Plates 16–19, and 1 Text-figure.

Contents.

"Ich hoffe zuversichtlich, dass wir nicht mehr allzu weit von dem Augenblicke entfernt sind, wann es klar werden wird, dass die verschiedenartigsten Angaben, insofern dieselben einer ernsten und gewissenhaften wissenschaftlichen Arbeit entspringen, alle in reinen Einklang gebracht werden, so dass ein neues schönes Gebäude, das der Bacterien-cytologie, in der allerfeinsten der Wissenschaften hoch emporragen wird."—Mencl (1910).

Introduction.

It is a remarkable fact that modern cytology, which has recently made such rapid strides as the result of the enthusiastic investigations of a vast army of workers, has almost lost sight of the Bacteria. Cytologists and protistologists alike have been content, for the most part, with assuming that the Bacteria are a group of simple organisms, possessing but little structural differentiation, and have then left them alone. Yet no biologist would deny, I think, that it is of the utmost importance that we should possess exact detailed knowledge of the structure and life-history of this immense group of living beings. More than one of the current conceptions in biology must undergo profound modification when we have precise information regarding the Bacteria.

If anyone endeavours, at the present moment, to ascertain from the vast bacteriological literature, which has been pouring out for many years past, the present state of knowledge regarding the structure of Bacteria, he will find that the whole matter is in a state of utter chaos. He will find that

the most divergent views are held regarding the various structures present in the bacterial cell. He will find, for example, regarding that most important of all cell-structures—the nucleus, that all views regarding its existence are held—from that which tells him that there is no nucleus of any sort, to that which tells him that the whole cell is to be regarded as a free nucleus.

Now the reason for this divergence of opinion is not far to seek. For many years the Bacteria have been entrusted to the bacteriologists, and only an occasional botanist or zoologist has ventured to poach on their preserves. Yet to the bacteriologists, the Bacteria are but a means to an end—they study them in order to cure a cold or make a cheese. Modern bacteriological methods are excellent and adequate when applied to medical diagnosis or industrial needs, but they are inadequate when applied to a study of the Bacteria themselves.[1] It is for this reason that professed bacteriologists possess such remarkably diverse opinions regarding the normal structure of Bacteria, and it is for this reason also that what little is definitely known of their cytology is due largely to the labours of a few zoologists and botanists. The bacteriologists are, of course, not to blame for this. Their aims are wholly different from those of the protistologist or cytologist. It is from these that our knowledge of the structure of Bacteria must come.

The great majority of Bacteria which have been described have taught us nothing concerning the internal structure of the bacterial cell. Nearly all the pathogenic forms are of exceedingly small size; and in addition to this great disadvantage they have mainly been studied after fixing and staining in the usual bacteriological manner, which renders them worthless for cytological purposes. It is desirable, in the first place, to study the largest and most easily investigated

[1] The truth of this can easily be seen by anyone who will consult the vast number of text-books on bacteriology which are in current use. In the majority of these, the cytology of Bacteria is not noticed at all, or else dismissed with a few inaccurate remarks made at random.

forms, and to examine them after treatment by suitable cytological methods.

The foregoing considerations have led me to a study of the cytology of the Bacteria. During the last four years I have devoted a considerable amount of time and labour in an endeavour to arrive at positive conclusions regarding the structure of the bacterial cell. It has been my object to discover large Bacteria which can be investigated cytologically with comparative ease—both whilst living and after suitable fixation and staining. The present paper represents the greater part of the results of my work, which—though still in progress—has led me to conclusions which are sufficiently definite to appear to me worth publication. I do not claim that the problem of the cytology of the Bacteria has been solved. My results are here given merely as a contribution towards a solution of the problem: I know only too well how incomplete and imperfect they are.

My main object has been to discover whether the Bacteria are nucleate or enucleate cells. It is useless to speculate upon the "simplicity," "primitiveness," "lack of differentiation," etc., which this important group is supposed to display, when such a simple point as this remains in doubt. I have endeavoured to find out whether a nucleus is present, and—if present—what form or forms it may assume. As staining reactions and micro-chemical tests appear to me to have been a signal failure in this direction, I have attacked the problem from another point of view—the morphological. I hoped—and I confess I am not altogether disappointed—that a study of the morphological elements present in the cell, and their behaviour during the various phases of the life-cycle, would throw considerable light upon the matter. Such results as I have obtained are, at least, very definite. They are, moreover, supported by the less important—as I believe—results derived from staining reactions.

As there is already a very extensive and confusing literature dealing with the structure of Bacteria, I have thought it advisable to give a brief historic review of the more important

work which has been done previously on the subject. I shall then give my own observations—recording them quite independently of the work of others—and reserve a full discussion of the whole matter to the final section of the paper.

My work was begun in the Zoological Laboratory in Cambridge. Afterwards I continued it whilst working in the Zoological Institute in Munich, and at the Zoological Station in Naples.[1] Subsequently I was able to add to my results whilst visiting Ceylon in 1909, during my tenure of the Balfour Studentship of Cambridge University. I have completed my work up to its present state at the Imperial College of Science and Technology, London. I desire here to record my indebtedness to all those who have—in one way or another—assisted in the furtherance of my work in the various places mentioned.

Historic.

In the pages which now follow, I have attempted to give a brief historic account of the most important work which has been contributed towards a solution of the problem of the nucleus in Bacteria. It is obviously impossible—in a paper of the present scope—to enter encyclopædically into all the work which has been done in this connection.

In dealing with the cytology of Bacteria, it is of the very greatest importance to consider the technique by means of which the various workers have reached their results. I shall therefore make a special point of noting in each case—wherever possible—the methods of fixation, staining, etc., which have been used. When this is done, it becomes apparent that a large part of what has been written upon the bacterial nucleus is practically worthless—owing to the inadequacy of the technique employed.

The older observers were mostly content to regard the Bacteria as enucleate—Monera, as Haeckel termed such

[1] Whilst occupying the British Association Table in 1908, under a grant from the Goldsmiths' Company.

supposed forms.[1] Early workers (e.g. Cohn) noticed, indeed, granular bodies in many Bacteria, but they were unable to reach any definite conclusions regarding their significance.

If we turn to older books on bacteriology, we find it usually stated that no nucleus is to be found in these organisms. De Bary (1884) says: "Nuclei have not yet been observed in Bacteria" (p. 492). Similarly, Zopf (1885) states: "Until now, nuclei have been looked for in vain in bacterial cells" (p. 14). Hüppe (1886), whilst pointing out that no nucleus had ever been shown to exist in Bacteria, suggested that the whole bacterial cell might be the homologue of the nucleus of other forms. This view has found many subsequent adherents.

One of the very first to investigate the structure of Bacteria was Kunstler (1887). He described in Spirillum tenue—after fixation with osmic acid, and staining with "noir Collin" or hæmatoxylin—an alveolar structure of the protoplasm, with numerous granules. In the later publications of Kunstler and his colleagues, descriptions which seem essentially similar are given of a number of different Bacteria. The descriptions are usually so incomplete, however, the figures usually so diagrammatic, and the technique employed usually so imperfectly indicated, that I find great difficulty in interpreting his results. (See Kunstler et Busquet [1897, 1898], Kunstler [1900], Kunstler et Gineste [1906, 1906A], etc.) As a rule, Kunstler appears to think that there is, in most Bacteria, no structure comparable with a nucleus.

Schottelius (1888) claimed to have found nuclei in various Bacteria (B. anthracis, cocci, etc.). These nuclei are said to be in the form of a short rod (bacilli) or spherule (cocci), and to divide in the process of cell-division. They are said to be visible in the living cells, but more distinct in dry films stained with gentian violet. The method of fixation is not given.

[1] It is perhaps worthy of note that, so late as 1894, it was still dogmatically stated by Haeckel that Bacteria contain no nucleus ('Systematische Phylogenie der Protisten und Pflanzen').

Babes (1889) found stainable granules—whose presence he had recorded at an earlier date—in various bacterial cells. Later (Babes, 1895), he named them "metachromatic granules," but he was unable to determine their precise significance.

Ernst (1888) found similar granules in the cells of Bacillus xerosis. They were observed in dry, flame-fixed cells, stained with methylene blue and Bismarck brown. He believed that they took part in spore-formation. Subsequently (Ernst, 1889) he found similar granules—using similar methods—in a number of other Bacteria. He proposed the name "sporogenic granules" for them, and regarded them as probably of a nuclear nature. Still later (Ernst, 1902), he described "chromatophil" granules—of uncertain significance—in many Bacteria (B. megatherium, water Bacteria, etc.). These granules were coloured by intra-vitam staining with methylene blue and neutral red.

The carefully conducted and classic work of Bütschli (1890, 1892, 1896, 1902) can here be considered in its main outlines only. After studying the Cyanophyceæ, Bütschli turned his attention to the large sulphur Bacteria.[1] In these he believes that the protoplasm, which has an alveolar or honeycomb structure, is differentiated into a peripheral layer and a denser "central body." In the meshes of the latter, granules which stain red with hæmatoxylin ("red granules") are present. He regards the "central body" with its "red granules" as the homologue of the nucleus of other cells, and the peripheral layer as the homologue of the cytoplasm. In the smaller Bacteria which he investigated, he found that the peripheral layer was relatively greatly reduced in size, or altogether absent—the greater part, or the whole of the cell being therefore constituted by the "central body." He was therefore led to regard the whole cell as homologous with a nucleus. The observations were made not only upon living cells, but also upon cells fixed, stained and variously treated by a number of different reagents.

[1] The earlier work of Winogradsky (1888) and others, upon this group, did little to elucidate the structure of the cells.

Wahrlich (1890, 1891) studying a number of different forms (*B. subtilis*, *B. megatherium*, etc.), arrived at conclusions essentially the same as those of Bütschli. He believed, from their chemical and staining reactions, that Bacteria contain chromatin. Young cells are homogeneous, chromatic; older cells show a reticulum of linin in which granules of chromatin are suspended. The chromatin granules fuse to form spores. He concludes that Bacteria are therefore really nuclei. All his work appears to be based upon a study of *dried* cover-slip preparations.

Zettnow (1891), using Löffler's flagellar stain—which has little value from a cytological point of view—agreed with Bütschli's conclusions regarding small Bacteria. Later (Zettnow, 1897) he extended his observations to large *Spirilla*, using chiefly *intra-vitam* staining with methylene blue, and drawing the same conclusions as before. Still later (Zettnow, 1899), he examined a number of Bacteria stained by Romanowski's method, but *after flame-fixation*. His conclusions regarding structure were essentially the same once more—that Bacteria consist entirely, or in some cases chiefly, of nuclear substance.

Protopopoff (1891) found granules which stain with fuchsin in a *Bacillus* from a cow's tongue, and in *Actinomyces*. He interpreted them as being of a nuclear nature, though on very slender evidence. The method of fixation is not stated.

Wager (1891) described a nucleus, containing two deeply staining rods and surrounded by a very thin membrane, in a *Bacillus* from the scum on water containing decaying *Spirogyra*. The division of the nucleus is briefly described. The method of fixation is not given, but it is stated that cover-glass preparations were stained with fuchsin. Wager (1895) again described structures which he believed to be nuclei in various other Bacteria, but gave only a very fragmentary account both of the structures themselves and of the technique employed.

Frenzel (1891, 1892) gives a description of several species of Bacteria—chiefly from a study of living cells—and draws analogy between spores and nuclei.

In 1892 Sjöbring described large vesicular nuclei, which divide by mitosis, in *B. anthracis*, hay Bacteria, the *Vibrio* of fowl-cholera and several micrococci. Fixation is stated to have been effected with nitric acid (alone, or with alcohol) without previous drying. The stains used were carbol methylene blue or carbol magenta.

Trambusti and Galeotti (1892) investigated a large *Bacillus* from water. The preparations were either dried, or fixed with HNO_3, and stained with safranin. The organisms stain at first uniformly, but later show a differentiation into darkly staining longitudinally placed rods, and granules. Subsequently young cells appear to be formed endogenously. The authors compare the structural changes with mitosis, though the reason for this is far from obvious.

Mitrophanow (1893) studied the structure of various sulphur Bacteria (*Beggiatoa*, *Chromatium*, *Ophidomonas*, etc.), also of *Cladothrix*, *Spirilla*, *Bacilli*, etc. He employed *intra-vitam* staining with methylene blue, and also examined organisms after fixation with various reagents and treatment with various stains. He believed that a nucleus was present in all the forms examined. Various modifications were described and figured. He did not agree with Bütschli's interpretation of the structures present in the bacterial cell. He believed "que toutes les bactéries que nous étudions ne peuvent être aucunement considérées comme des organismes sans noyau ; de même on ne peut pas leur attribuer exclusivement une nature de noyau. Elles apparaissent commes des cellules dans divers stades de complication, laquelle est exprimée par la séparation plus ou moins complète du noyau."

Podwyssozki (1893) gives an account of the structure of the cholera *Vibrio*, as seen in dried preparations stained with Ziehl-Neelsen and in cells treated simply with fuchsin. He finds a nucleus-like oval mass of "chromatin" in the cell, and other bodies of different (undetermined) nature. In place of the oval mass of chromatin, two or more masses may sometimes be seen—appearances which he regards as due to degeneration.

Schewiakoff (1893) finds a structure like that described by

Bütschli in sulphur Bacteria, in a large freshwater organism which he names Achromatium oxaliferum. This organism resembles the sulphur Bacteria in general form, but contains calcium oxalate—probably in combination with a carbohydrate—instead of sulphur. There is a "central body" present, containing colourable granules which undergo division.

Ilkewicz (1894), studying B. anthracis after flame-fixation and a complicated staining process, found darkly staining bodies present, which he believed to be spore-rudiments. He suggests that it is these structures which Sjöbring mistook for nuclei.

A. Fischer (1894) explains the protoplasmic differentiation described by Bütschli as due to plasmolysis. In this, as in subsequent memoirs (Fischer, 1897, 1899, 1903), he maintains that a "central body" does not exist: that the granules are probably reserve material, and neither nuclei nor chromatin: and that no nucleus has been demonstrated in Bacteria. The cell is not the equivalent of a nucleus. His conclusions are based upon elaborate studies of fixation and staining methods. It is hardly necessary to enter here into the polemics which have taken place between Fischer and Bütschli.

Migula (1894), after a study of Bacillus oxalaticus, reaches the conclusion that no "central body" is present in this form. Colourable granules—insoluble in pepsin—are present, but no definite interpretation of them is given. In a subsequent work (Migula, 1897), after reviewing the literature he concludes: "Ueber die Bedeutung der Körnchen in der Bakterienzelle lassen sich nur sehr subjektive Vermutungen hegen; ich möchte sie als die ersten Anfänge einer Zellkernbildung betrachten." More recently (Migula 1904), he expresses the opinion that the existence of a nucleus is still an open question.

That nuclear structures occur in many Bacteria is believed by Löwit (1896). His conclusions are based, however, upon dried preparations stained with Löffler's flagellar stain.

A. Meyer (1897), using various methods, found granules which he interpreted as nuclei in B. asterosporus and B.

tumescens. In a later paper (Meyer, 1899) he extended these observations to a number of other Bacteria. He employed various methods—chiefly fixation with formol and staining with fuchsin. The granules, which are nuclei, may be from one to six in number in each cell. In 1904 he gave a detailed account of the chemical and staining reactions of "volutin" granules in Bacteria and other organisms. More recently (Meyer, 1908) he affirms that his "nuclei" are not volutin, but condemns the nuclear structures described by the majority of other workers.

Wagner (1898) discovered a nucleus in the form of a granule, dividing with a dumbbell figure—one in every cell —in B. coli and B. typhosus. His preparations were "dried in the usual way" and stained by a very elaborate method.

"Chromatin" bodies were found in various forms of Bacteria by Ziemann (1898). He made dry films, fixed in the flame or in alcohol, and stained by Romanowski's method.

Macallum (1899) investigated three species of Beggiatoa, after various methods of treatment. He finds no such differentiation as described by Bütschli. Compounds of masked iron and organic phosphorus are uniformly diffused through all the protoplasm, and these compounds also occur in certain granules which stain with hæmatoxylin. "There is no specialised chromatin-holding structure in the shape of a nucleus of any kind."

Rowland (1899) records the results of staining various Bacteria—chiefly with roseine, without fixation. Deeply stainable granules were found, though no very definite interpretation was given to them. He appears to think that they may be partly nuclear and partly excretory.

Under the name Bacterium gammari, a large nucleate organism—inhabiting the body cavity and hæmolymph of Gammarus zschokkei (from Garschina Lake, Switzerland) —was described by Vejdovský (1900). The organisms were treated by various cytological methods. Each cell has a

distinct nucleus lying towards the centre. Later (Vejdovský, 1904), he describes stages in the mitotic division of this nucleus, and records similar nuclei in certain filamentous Bacteria inhabiting the gut of Bryodrilus ehlersi.

Marpmann (1900) suggests—amongst other things—that enucleate Bacteria may exist. His observations are very fragmentary, and all made upon flame-fixed organisms.

Feinberg (1900) describes "nuclei" of various forms in various species of Bacteria (B. coli, B. anthracis, Micrococci, etc.). The observations were made upon organisms stained by Romanowski's method. The method of fixation is not given; presumably the preparations were dried and flame-fixed. (See here also Zettnow, 1900.)

Marx and Woithe (1900) arrive at the conclusion that the Babes-Ernst granules afford an index of virulence—greater numbers indicating a greater degree of pathogenicity. They further state that the organisms containing these granules are the "Träger und Erhalter der Art." They also make the statement that "the Babes-Ernst granules are products of maximal condensation and typical localisation of the enchromatic substance of the bacterial cell." The illuminating nature of such a statement is obvious. Regarding the relation between the granules and virulence, the statement of Marx and Woithe has been controverted by Ascoli (1901), Gauss (1902), Schumburg (1902), Krompecher (1901), Ficker (1903), Guilliermond (1906), and others.

Krompecher (1901), working on various organisms, draws a distinction between "metachromatic granules" and "Babes-Ernst granules," on the grounds of staining reactions. He leaves the significance of the granules in doubt. (See here also Mühlschlegel [1900], Marx [1902], etc.)

Hinze (1901) found scattered granules, which he believed to be chromatin, in the cells of Beggiatoa. Later (Hinze, 1903), he described similar bodies in another large sulphur bacterium—Thiophysa volutans. Various methods of fixation (Flemming, etc.) and staining (Heidenhain, etc.) were employed. The granules are said to divide by a process

of simple constriction. An ordinary form of nucleus was not found.

Nakanishi (1901) describes nuclei in a large number of Bacteria (Cocci, Bacilli, Spirilla) stained with methylene blue, either intra-vitam, or after fixation with formol vapour. He finds minute spherical nuclei in Cocci; nuclei in the form of a granule, rodlet, or filament in Bacilli; and granular or filamentar nuclei in Spirilla. He also finds nuclei in spores. He gives an excellent account of his technique, good figures, and strong evidence for the conclusion that the structures he observed are really nuclei. His interpretations have been unfavourably criticised by Ascoli (1901 A), Ficker (1903), Preisz (1904), Meyer (1908), and others.

Schaudinn (1902) inaugurated a new era in bacteriology by studying cytologically the whole life-cycle of the gigantic Bacillus bütschlii in the gut of the common cockroach. He described a nucleus in the form of scattered granules of a chromatic substance (chromidia) throughout the greater part of the life-cycle. During spore-formation the granules arrange themselves in a spiral and finally become aggregated into dense masses in the fully formed spore. A process interpreted as a modified sexual act (autogamy) was discovered. In the following year (Schaudinn, 1903) he described analogous conditions in Bacillus sporonema, a small marine organism.

Meyer's pupil Grimme (1902) has given a lengthy and elaborate account of the chemical and staining reactions of many different kinds of granules which occur in many different Bacteria. After a discussion of the various kinds of granules which he studied—especially the "metachromatic granules" ("Volutanskugeln")—he finally decides in favour of the nuclear views of Meyer. The "nuclear" granules of most other observers are probably not nuclei. (In connection with these granules see also Guilliermond [1906, 1910, etc.], Meyer [1904], Eisenberg [1910], etc.).

Under the name Spirillum colossus, Errera (1902) describes an enormous spirillar form. Darkly staining masses

of variable form are seen in dried and stained preparations. Their interpretation is not indicated. (This organism is certainly worthy of a careful cytological study.)

Federowitsch (1902) studied B. megatherium, B. pyocyaneus, and other Bacteria. He found stainable granules, which play a part in spore-formation, in the cells. But he believes that "no nucleus like that of higher cells" is present. The method of fixation is not given; Weigert's stain was employed.

Růžička (1903) finds granules present in many Bacteria after fixation with $HgCl_2$ and staining with methylene blue. A definite interpretation is not given to the granules. In later papers (Růžička, 1908, 1909, etc.) he advocates the view that the bacterial cell represents a naked nucleus.

Ficker (1903) discusses the problem of the nucleus in Bacteria. He expresses the opinion that it is premature to draw any conclusions with regard to either granules or nuclei.

Mencl (1904), using careful cytological methods, finds typical nuclei in Bacilli inhabiting the gut of the cockroach. He also finds nuclei in B. megatherium. In 1905 he describes nuclei of many different forms in filamentous water Bacteria (Cladothrix, etc.), after staining intra-vitam with polychrome methylene blue. Later (Mencl, 1907) he gives a minute description of Bact. gammari, describing the various appearances seen in resting and mitotically dividing nuclei. He also published in the same year (Mencl, 1907A) a more detailed account of the symbiotic Bacteria of the cockroach. Quite recently he has given a description of the nuclei in Sarcina and Micrococci as revealed by staining with polychrome methylene blue intra-vitam and subsequently clearing in glycerine. Mencl's results have been adversely criticised by Guilliermond (1907, 1908, 1910) and Meyer (1908). The latter states that Mencl's nuclei are really volutin granules; the former believes they are the septa formed in the cells during cell-division. Mencl (1909) has replied to Guilliermond's criticisms and maintains the correctness of his own interpretations.

Dietrich (1904), after reviewing the literature on the subject, says: "Wir wollen nur noch als Hauptergebnis betonen, dass alle Versuche, Kerne in Bakterien zu finden, als gescheitert zu betrachten sind."

Preisz (1904) gives an elaborate account of the structure of the anthrax Bacillus. He studied the organisms after mixing them with alcoholic fuchsin, formol-fuchsin, or methylene blue. He maintains that the nuclei described by Schottelius, Nakanishi, etc., are really more deeply coloured portions of the cytoplasm. The real nuclei are in the form of minute spherical corpuscles, one or more in each cell. They undergo division. They are distinct from the metachromatic granules of Babes and Ernst, and from the acid-fast granules of Bunge. A nucleus enters into each spore. He finds similar nuclei in B. cohærens, B. tetani, and B. asterosporus. His conclusions are therefore essentially the same as those of Meyer. (Cf. here also Georgevitch [1910].)

Raýman and Krnis (1904) describe typical nuclei—similar to those found by Vejdovský and Mencl—in a variety of Bacteria (B. mycoides, B. tumescens, etc.). They are found in young cells only. The method of treatment is peculiar—fixation by desiccation (in a desiccator) and staining with iron-hæmatoxylin and purpurin. Excellent photo-micrographs are given. The conclusions of these investigators are challenged by Guilliermond (1908).

Swellengrebel (1906) records the results of a minute cytological and micro-chemical investigation of Bacillus maximus buccalis. He finds a nucleus present in the form of a more or less complete spiral or zig-zag filament. In the following year (Swellengrebel, 1907), he describes two large spherical nuclei in Bacterium binucleatum—an organism from the human mouth. He also describes spiral or zig-zag nuclear filaments or rodlets in Spirillum giganteum (Swellengrebel, 1907A), and subsequently (1909A) in certain filamentous Bacteria (Sphærotilus, Thiothrix). His results have been questioned by Hölling (1907), Zettnow

(1908), and Guilliermond (1908). The various objections raised against his work have been answered by Swellengrebel (1908, 1909), who maintains the correctness of his conclusions.

Guilliermond (1907) gives an excellent brief review of previous results upon the cytology of Bacteria. In the following year (Guilliermond, 1908) he describes the structure of a number of Bacilli (B. radicosus, B. mycoides, B. megatherium, etc.). He believes that in all these the nucleus is in the form of granules of chromatin (chromidia)—distinct from the metachromatic granules—scattered through the cytoplasm. These granules become massed together to form the spores. He criticises the results obtained by many other investigators. Various cytological fixing and staining methods were employed in his researches. In a more recent paper, Guilliermond (1909) describes nuclei in the form of spiral filaments—like those found by myself—in two species of Bacillus (from the gut of Echinocardium) and a large Spirillum.

In 1908 I gave the results of cytological researches which I had undertaken upon the structure and life-history of several Bacteria. I described a new large disporic Bacillus —B. flexilis, from the gut of frogs and toads—whose life-history is essentially the same as that of B. bütschlii described by Schaudinn (1902). I also described another organism—which I named Bacillus spirogyra—from the same hosts, in which the nucleus is in the form of a spiral or zig-zag filament. I described further in Spirillum monospora—from the frog and toad also—a nucleus of the chromidial form. The chromidia mass themselves together in forming the spores. In 1909 I gave a more detailed description of B. spirogyra. I discussed the nature of the nuclear filament, and described the part it played in spore-formation—a process which I described in detail. I descibed in addition the structure and method of spore-formation in B. lunula, which resembles in these respects B. spirogyra, As a result of this work, I reached the conclusion that the

"autogamy" of B. bütschlii (Schaudinn, 1902) and B. flexilis was probably not a sexual process at all.

Amato (1908) describes results obtained by staining several Bacteria (B. mycoides, Sp. volutans, etc.) intra-vitam with Brillantcresylblau. He believes that in the spore, and at the beginning of development, a relatively large spherical nucleus is present, which breaks up subsequently into chromidia. The divergent views of different observers may have an explanation in the fact that they observed similar organisms, but at different stages in their development.

Dangeard (1909) records the results of a re-investigation of Chromatium. By fixing with Flemming or Perenyi, and staining with various stains (especially Flemming's triple), he confirms the description of this organism given by Bütschli. A "central body" corresponding to a nucleus is present. Additional evidence for regarding the "central body" as a nucleus is afforded by the fact that a rhizoplast can sometimes be seen connecting the flagellum with this body.

Ambroz (1909) gives a lengthy description of Bacillus nitri. As a result, he reaches the same conclusion as Růzicka —that Bacteria are nuclei. Fixation is said to have been effected with a concentrated solution of "HCl_2,"[1] and staining chiefly with Giemsa.

Under the name "Hillhousia" mirabilis, West and Griffiths (1909) describe a very large sulphur bacterium. There is said to be a protoplasmic network present, containing granules believed not to be chromatin. "Nothing of the nature of a definite nucleus is present." Details—especially as regards the method of using formol as a fixative—are too scanty for this conclusion to be accepted without further evidence. No reference is made to the work of Bütschli, Schewiakoff, Hinze and others, on similar forms.

Recently, an account of the structure of the long forms of B. coli, B. typhosus, etc.—produced by growing these organisms on culture media containing aniline dyes—has been

[1] I presume this means $HgCl_2$,— and not HCl, as given by Guilliermond in a review of this paper in 'Bull. Inst. Pasteur.'

given by Vay (1910). He finds large irregular masses of darkly stained substance—which he calls chromatin—in these organisms. He does not appear to be aware that the production of these forms on coloured media had already been described by Walker and Murray (1904).

Such, then, is a very condensed account of the chief work which has hitherto been published concerning the problem of the nucleus in Bacteria.

In all work in which inadequate technique has been employed—for example, in all studies in which only dried and flame-fixed organisms have been examined—the conclusions attained can have little value from a cytological point of view. In many publications, moreover, the descriptions both of results and of methods are so meagre as to render discussion of them either unprofitable or impossible. Therefore I shall—on either or both of these grounds—eliminate the following works from any further discussion :

Kunstler and Busquet (1897, 1898), Kunstler (1900), Kunstler and Gineste (1906, 1906A), Schottelius (1888), Zettnow (1891, 1899), Protopopoff (1891), Wager (1891, 1895), Sjöbring (1892), Trambusti and Galeotti (1892), Ilkewicz (1894), Löwit (1896), Wagner (1898), Ziemann (1898), Marpmann (1900), Feinberg (1900), Errera (1902), Federowitsch (1902), West and Griffiths (1909), Vay (1910).

I think most cytologists will agree with me that no profitable discussion of these papers is possible.

Material and Methods.

As I have already indicated above, I have made a special point of working upon the largest forms of Bacteria which I have been able to find; but I have studied in addition a number of small forms, when they have been suitable.

Small Bacteria are not only very difficult to investigate on account of the limitations imposed by the microscope, but they are also in many cases unsuitable in other ways for cytological study. They occur frequently in media which render

the making of good microscopical preparations exceedingly difficult and laborious, and they contain granules (reserve material, etc.), which are relatively of such a size as to obscure much of the structure of the living substance itself. For the latter reason, the sulphur Bacteria, in spite of their large size in many cases, appear to me to be unfavourable objects for study—as a starting-point, that is to say, on our way to a comprehension of the organisation of Bacteria.

Another point that has seemed to me of some importance is this. Much of the work which has been done upon the structure of Bacteria has been based on a study of organisms which have been kept in cultures for a greater or less period of time. It seems to me highly probable that the discordant results of different workers may in many cases be due to cultural differences in the organisms studied. Different culture media may be used in the cultivation of Bacteria: but although "pure" cultures may be obtained in half a dozen of these, it does not follow that all or any of the colonies so obtained consist of normal individuals. Bacteria are not found in nature as a rule in pure cultures, and this is a point which should not be overlooked when considering their normal structure. Culture methods are of the greatest service in the separation of various microbes from one another, but it does not at all follow that all pure cultures of a given organism are identical, or that they contain individuals which are in every way the same as those living in their natural environment. I have therefore not studied Bacteria grown in artificial culture media, but have confined my attention for the present to organisms in their natural habitat. The fact that the Bacteria which I have investigated are not—for the most part—previously described and named "species" from pure cultures, is therefore not an objection which can be urged against my results, but a necessary consequence of the point of view from which I have attacked the problem.

As a source of material, I have found the intestinal contents of various animals most useful. The contents of the large intestine in many animals is swarming with Bacteria, frequently

of large size The consistency of the intestinal contents, moreover, is usually such as to render the making of microscopic preparations (smears, etc.) comparatively easy. I have found the contents of the large intestine of Amphibia and Reptilia especially suitable: but insects, mammals and other animals also contain a rich supply of suitable material which is as yet almost untouched. Most of the organisms which I am about to describe have been obtained from frogs, toads and lizards.

I have found in all the animals which I have studied that the Bacteria in the large intestine vary enormously—in different individuals—both as regards the number of different forms, and the number of microbes as a whole. In the frog, for example, some individuals may contain very few Bacteria—mostly of the same form—whilst others may contain countless numbers of Bacteria of the most diverse forms. This is, of course, only what one would expect.

As the source of the material will be found under the description of each organism, I shall here say nothing more detailed regarding this, but will now devote a few words to a description of the technique which I have employed in my researches. I have already (Dobell, 1908) given a brief account of some of the methods which I have used.

I have tried most of the methods of fixation and staining which are usually employed in cytological work. It is usually necessary to modify the ordinary procedure in one way or another when dealing with Bacteria. In my experience, the usual methods of fixation (e.g. corrosive sublimate, Flemming's solution, Hermann's solution, osmic acid, formalin, various picric acid and bichromate solutions, etc.) may all—under suitable conditions, and with careful procedure—be made to give excellent results. Fixation is most easily and effectively accomplished by making a wet film of the intestinal contents—or other medium in which the Bacteria occur—on a coverslip, and then dropping it film-side downwards upon the fixing solution. Drying previous to fixation is, of course, to be avoided. The usual bacteriological method

of making dry films and fixing them by passing them through a flame is quite worthless from a cytological point of view, owing to the plasmolysis and distortion which it brings about.

When the medium containing the Bacteria is too watery to allow of fixable films beeing made, gelatine or albumen may be added until a film of suitable consistency is obtained. If the medium be too thick, one must of course be careful to use isotonic salt solutions for its dilution.

Most of the ordinary cytological stains (e.g. Delafield's hæmatoxylin, carmine, safranin, etc.) I have found unsuitable for Bacteria. They—like most of the ordinary aniline derivatives—are liable to stain the whole cell uniformly, without differentiating the internal structures. This is largely due to the marked affinity which the cell wall has for many stains, causing it completely to obscure the finer structures present in the protoplasm.

After trying a large number of combinations of fixatives and stains, I have latterly confined myself almost entirely to two methods. Both of these have proved of the greatest value. They are (1) fixation with osmic acid or formalin, followed by staining with one of the modifications of Romanowski's method, and (2) fixation with Schaudinn's sublimate-alcohol (2 : 1) followed by staining with Heidenhain's iron-alum hæmatoxylin. The latter method is now so well known (see, for instance, Schaudinn, 1902) that I will not re-describe it. It is of course a wet film method, and its only disadvantage is that it is exceedingly difficult to use, owing to the difficulty of obtaining exactly the right degree of differentiation. Indeed with different degrees of differentiation quite different appearances may be produced in the same Bacteria, and it is therefore necessary to be very cautious in interpreting the results. Nevertheless, I believe this method to be one of the most valuable for the study of the structure of Bacteria.

With regard to the first method, I have found it so simple and easy to use that I can strongly recommend it to others. My method of procedure is as follows. I take a drop of the medium containing the Bacteria and place it in the centre of

a carefully cleaned glass slide (or coverslip) by means of a platinum loop. I then place a drop of 1 per cent. osmic acid or strong formol (40 per cent. formaldehyde, Schering) beside the first drop, and then mix both together and spread the fluid in a thin and even film on the slide. I then allow the film to dry, which usually takes a few minutes. No heating should be used to accelerate the process. The slide or coverslip with the dried film is then placed in absolute alcohol for about ten to fifteen minutes. It is then removed, and the film allowed to dry once more. I then stain the film with Giemsa's or Leishman's stain in the usual way. After staining I differentiate in 30 per cent. alcohol—wash in distilled water—dry by blotting with a cigarette paper—and mount in cedar wood oil or neutral Canada balsam. Chromatin structures are coloured a bright red; the cytoplasm being blue, lilac or pink, according to the degree of differentiation. The structure of many Bacteria is revealed with remarkable distinctness by this method—its chief disadvantage being that the preparations sooner or later fade, and cannot as a rule be satisfactorily re-stained.

The above method of fixation—which I term the drop method—calls for some further comments. In the first place, it might be urged that the drying which takes place would be liable to injure the organisms, and give rise to misleading appearances. This is not so, however. If the Bacteria are fixed with osmic acid or formol before drying is allowed to take place they are not plasmolysed or injured in any way. It is only when drying takes place before fixation that such disastrous results ensue.

I have made many preparations by other methods as controls. I have made wet films and fixed them by immersion in 1 per cent. osmic acid or formol: I have also made wet films and fixed them by exposure to osmic vapour: and I have then stained these films by modifications of Romanowski's method and mounted them in balsam without allowing any drying to take place at any stage in the proceedings (cf. Dobell, 1908). The final results obtained in all these cases are almost in-

distinguishable from one another. The only real difference observable is that the organisms which have been dried appear slightly broader than those which have not—owing to the slight flattening which drying brings about. The internal structures appear exactly alike. Controls with wet films fixed with sublimate-alcohol and stained with Heidenhain's iron-hæmatoxylin give confirmatory results. I therefore think that the drop method of fixation, when employed in the manner described, gives reliable cytological results in the case of Bacteria.[1] On account of the ease with which osmic acid or formol may be employed in this manner, I have used them more frequently than any other fixatives.

Another point which calls for comment concerns the use of alcohol after fixation. When "osmic acid" (more correctly, osmium tetroxide, OsO_4) is used—either in solution or in the form of vapour—it is, of course, unnecessary to treat the preparations subsequently with alcohol—so far as complete fixation is concerned. In practice, however, I find that films fixed by osmic vapour or by the drop method adhere to the slide or coverslip better if they are hardened in absolute alcohol for a short time after fixation. When formol is employed, however, it is absolutely necessary to employ alcohol subsequently. As is well known, formaldehyde fixes protoplasmic structures without precipitating them in an insoluble form. It is therefore necessary to place the fixed structures in strong alcohol before proceeding further—otherwise fixation may be completely undone in subsequent treatment.[2]

[1] I may add that beautiful preparations of small flagellates and other Protista may also be obtained in this way.

[2] Cf. Gustav Mann (1902). This point seems worthy of attention. I note that Swellengrebel (1906) fixes Bacteria by the drop method, using formalin. But he does not appear to use alcohol subsequently, so that many of the appearances which he describes may be due to imperfect fixation. If it is desired to use formalin alone—without using alcohol at all—and to use stains in watery solution, the fixation may be preserved by adding a small percentage of formalin to all the stains, etc., employed after the original fixation. If it is desired to dilute the formalin used in fixation, this should be done with isotonic salt solution—not with water.

Sometimes excellent results may be obtained by making ordinary dry films, fixing with absolute alcohol, and staining in the usual way with Giemsa or Leishman. This method is not to be relied upon, however, and should never be employed alone. Giemsa's new wet method (*vide* Giemsa, 1909, 1910) appears to give excellent results, but I have not used it myself for Bacteria.

I have employed *intra-vitam* stains in many cases, but with little success—so far as nuclear structures are concerned. I have used neutral red, Brillantcresylblau, and methylene blue. Many other workers appear to have been more successful with these stains (*e.g.* Mencl, who has obtained most striking results with polychrome methylene blue). In my experience, only non-living structures in the cells (metachromatic granules, etc.) can be stained during life. But doubtless much depends upon the stain itself. Different samples of methylene blue—for example—may give quite different cytological results.

Descriptions of the Forms Investigated.

Having already given as briefly as possible the most important results which have been reached by previous work on the cytology of Bacteria (see p. 399), I shall pass on to a detailed description of my own observations. In this description I shall make no attempt to compare or to correlate my own results with those of others—my object being to give only the facts which my own work has disclosed. A discussion of all the results—obtained by other workers and by myself—will be reserved for a subsequent section of the paper (see p. 462, *et seq.*).

In describing the various forms which I have investigated, I have—for convenience—divided the organisms into five main groups. These are the Cocci, Bacilli, Spirilla, "fusiform Bacteria," and a group of other organisms which resemble—but are not—non-motile rod Bacteria. I shall deal with each of these groups separately, and in this order.

I. Coccus Forms.

Cocci of various sizes are very common in the large intestines of many different animals. Unfortunately, however, they are usually of very small size, and hence exceedingly difficult to study accurately. I have examined many cocci from the large intestines of frogs and toads (Rana temporaria, R. esculenta, Bufo vulgaris), of newts (Triton vulgaris), of cockroaches (Periplaneta americana and Stylopyga orientalis) and of several different snakes. All these have proved to be of little value, because the organisms were usually so small that I could not be certain of their structure as seen under the microscope. The living organisms were usually very refractile, and showed no internal structure which could be definitely separated from appearances due to optical phenomena. For instance in a small Micrococcus—examined under a high power—a dark spot of varying size can often be distinctly seen in the centre of the organism.[1] This is not, I believe, a definite body—such as a nucleus—lying in the cell, but is merely an appearance caused by optical phenomena connected with the microscope.

(A) Micrococci.

Only two Micrococci of suitable size for investigation have come under my notice, but they have both revealed a structure which is quite unmistakable. Both forms were found in the large intestines of lizards—Lacerta muralis and Mabuia carinata.

Micrococci from Lacerta muralis.

The lizards were obtained in the neighbourhood of Naples. I found that nearly all of them harboured a large Micrococcus in greater or less numbers.

[1] These appearances probably led Schottelius (1888) to believe that he could see a nucleus in living Bacteria.

The living Micrococci, examined in the contents of the large intestine immediately after removal from the lizard, showed no very definite structure. I have been unable to convince myself of the presence of any internal structures from an examination of living organisms alone.

With stained preparations, however, the case is very different. I have obtained the best results after fixation with 1 per cent. osmic acid or formalin, and after staining with Giemsa's or Leishman's stain in the manner already described (see p. 415). The following descriptions apply to organisms treated in this manner.

The Micrococci occur singly, in pairs, or in chains. They are usually perfectly spherical, and have a diameter (in fixed and stained specimens) varying from rather less than 1 μ, up to 2 μ. All intermediate sizes may be found. It is possible, of course, that the different sized forms are really different species. They all occur together, and in company with many other forms. But it is quite immaterial, for my purposes, whether they are one species or one hundred, for they all show a structure which is the same in each individual, and it is with their structure that I am concerned.

Every individual, after fixing and staining (cf. Pl. 17, fig. 45), shows a uniformly coloured cytoplasm, a well-marked cell wall, and a centrally situated, darkly staining body. This central body is always present. It is roughly in the form of a spherical granule, but may appear more or less square or triangular in optical section. It always takes up the chromatin stain strongly.

Among the ordinary "resting" forms just described, a number of dividing organisms can usually be found. The details of the process of division can be followed in stained specimens with great clearness, and present features of considerable interest.

Division—which results in the formation of two equal daughter-cells—takes place as follows (see figs. 46–49). In the first place, the central body becomes elongated—the cell itself also exhibiting a slightly rod-like form—and assumes a

characteristic dumb-bell shape (fig. 46). The long axis of the dumb-bell coincides frequently with the long axis of the cell, but it is also often seen to be slightly displaced from this axis —occupying a somewhat oblique position in the cell. The ends of the dumb-bell separate from one another, but remain attached by the slender intermediate strand for some time. When the central body has reached this stage, a constriction appears in the middle of the cell in a plane at right angles to the long axis of the dumb-bell figure. The cell now presents the appearance shown in fig. 47 (Pl. 17). A little later the ends of the dumb-bell lose their connection with one another, through the disappearance of the connecting strand. The constriction of the cell wall is now more marked (fig. 48). After the two new central bodies have been formed in this way from the original body, the cytoplasmic constriction becomes complete, and two daughter-cells are formed which lie at first in close contact with one another (fig. 49). In this Diplococcus-condition the daughter-cells may remain; or they may separate forming two free Micrococci; or they may divide again, and so give rise to a chain of coccus forms. Division always takes place in the manner just described—the central body dividing with the formation of a characteristic dumb-bell figure, and being followed by the fission of the cytoplasm.

Now I think there can be little cause for complaint if I call the central deeply staining body a nucleus. This body is a constant morphological feature of every cell: it divides with the formation of figures which are closely comparable with those of a very simple amitosis—on a very small scale: and is takes up the nuclear stain strongly. I shall discuss this more fully in a later part of the paper, and will henceforward call the central body the nucleus.

As I have pointed out above, the dividing nucleus not uncommonly occupies a slightly oblique position in the cell. It also shows occasionally another modification, which is of the greatest interest—a modification which is characterised by the dividing nucleus assuming the form of a zig-zag

filament. This condition may be more or less strongly marked: it may take the form of a simple bend, or it may take the form of a spiral filament consisting of one or more turns (see fig. 52).

It might be urged that the bilobed cells which contain a zig-zag, bent, or spiral filament are really different organisms from those under consideration. The proof that this is not the case lies in the fact that all stages can be found together in the same chain of organisms (fig. 52). There can be very little doubt that these chains are formed from the successive divisions of an originally single Micrococcus. In the short chain depicted in fig. 52, a pair of such forms is seen at the lower end of the chain. Above these, four dividing cocci are seen which show various modifications of the dividing nucleus, from a slightly distorted dumb-bell figure to a zig-zag or spiral filament.

I regard this configuration of the nucleus as of considerable significance. The matter will be discussed at greater length in a subsequent section of the paper (see p. 471).

Cocco-bacillar Forms from Lacerta muralis.

Now in addition to the coccus forms which I have just described, there are many organisms which cannot be very definitely classified with either coccus forms or bacillar forms, but which occupy an intermediate position. These forms (fig. 50) present the appearance of a slightly elongated sphere, or of a very short rod with rounded ends. The shortest, most spherical forms (fig. 50, upper right-hand individual) have a nucleus which is in the form of a short and usually bent rodlet. The longest forms (fig. 50, lower and upper left-hand individuals) show a nucleus which is in the form of a filament arranged in a more or less zig-zag or spiral manner. That such organisms are in a "resting" (i.e. not dividing) state, appears certain from the fact that no cytoplasmic constriction can be seen (compare figs. 45–50 and 52).

As I have already noted, the ordinary Micrococcus

forms show slight irregularities in the contour of the nucleus; and it is, in fact, frequently impossible to decide whether an individual should be described as a Micrococcus or a cocco-bacillar form. All intermediate gradations occur, so that—although an absolute proof is lacking—I believe that all these forms, from typical Micrococcus to typical Bacillus, are really stages in the life-cycle of one and the same organism.[1] For the present, however, I will confine myself to describing the morphological features of these forms—merely pointing out that, side by side in the same host, all forms occur from typical, spherical cocci with a spherical nucleus, to typical rod-shaped bacilli with a zig-zag or spiral nuclear filament.

Micrococci from Mabuia carinata.

These Micrococci were obtained from the large intestine of the Brahminy lizard (Mabuia carinata), caught in Ceylon (Colombo). They are of smaller size than those just described, and I have examined, relatively, only a small amount of material.

The organisms (Pl. 16, figs. 42–44) have an average diameter of about 1·5 μ, or rather less. They are spherical, and show a centrally placed nucleus just as in the case of the Micrococci from Lacerta muralis (cf. fig. 44). The method of division appears to be exactly the same, and I have therefore not figured it in detail. Allowing for the difference in size, figures 46–49 would be equally good representations of the dividing individuals of this form.

Coccus or cocco-bacillar forms in the gut of M. carinata also show the zig-zag form of nucleus (fig. 43). I have not, however, a complete set of stages between cocci and bacilli, as in the case of the Bacteria from Lacerta muralis.

These Micrococci do not present any other features of special interest. I have described them because they are the only other cocci which have furnished me with unequivocal evidence regarding their cytology.

[1] For further consideration of this see p. 484.

(B) Sarcina.

After investigating the structure of the ordinary Micrococcus forms, I naturally became curious to see what sort of structures could be found in the Sarcinæ. For some time I endeavoured to ascertain the exact structure of a Sarcina which is very common in the English frog and toad, but I was unable to reach any definite conclusions owing to the very small size of the individual cells. Other Sarcinæ from other animals proved equally difficult, but at last I discovered a large and suitable form in the large intestine of a Ceylon toad. This organism I will now describe.

Sarcinæ from Bufo melanostictus.

These Sarcinæ were obtained from a single toad which was captured near Colombo. All the preparations were made by fixing in 1 per cent. osmic acid and staining with Giemsa's stain. The following description therefore applies to organisms treated in this manner.

Sarcina is, of course, simply a colony of cocci, arranged typically in groups of eight individuals in three dimensions of space. The groups originate by the successive "cleavages" —like a developing egg—of a single coccus cell.

The individual cocci which compose the cell-groups of the Sarcina under consideration are of very large size. They measure on the average a little over 2 μ in diameter—some cells attaining a diameter of 2·5 μ.

In the living organism, it can be seen that nearly every cell contains one large refractile granule. This is probably reserve material of some sort. Sometimes this granule may be absent and occasionally two such structures are to be seen. No other internal structures can be made out with certainty in the fresh state.

Upon staining the organisms, however, the structure of the cell can be readily demonstrated (see figs. 24–29, Pl. 16). The cytoplasm appears a uniform blue,[1] and sometimes shows a

[1] Or pink, if the blue is extracted with alcohol after staining.

faint granular or alveolar structure. The refractile granules remain unstained, or after prolonged staining may take on a faint yellowish-pink tinge. In each cell a dark red granule—corresponding with the nucleus described above in Micrococci—can always be found. The position of this nucleus in the cell varies. It does not always lie in the centre, but is usually near this point, and very often in contact with the refractile granule (cf. figs. 24–29).

In resting cells, the nucleus has always this form of a simple granule. This is seen in fig. 24, which shows a two-cell stage. Division of the nucleus precedes the division of the cytoplasm, and is effected in the same way as the nuclear division of the Micrococci described above. The granule elongates slightly, assumes a dumb-bell figure, and then separates into two daughter-granules. Fig. 27 shows a three-cell stage, in which the two daughter-cells on the left have completed division, whilst the nucleus of the cell on the right is dividing. Fig. 26 shows a later stage. The two cells on the left contain dividing nuclei, whilst the single cell on the right contains two daughter-nuclei—cytoplasmic fission having not yet occurred. Fig. 28 shows a four-cell stage, each cell containing a resting nucleus. In fig. 25, one of the nuclei (upper left-hand cell) has divided into two, and in fig. 29 three out of the four cells show dividing nuclei. The eight-cell stage which results from the division of these four cells shows exactly the same sort of nuclei.

Judging from the large number of cells which showed dividing nuclei, I should think that cell division takes place very slowly in this organism, but I made no observations on this point on the living organisms.

It will be apparent, I think, to anyone who will compare the figures of the Sarcina with those of the Micrococci, that the structure of the cell and its nucleus—both during rest and during division—is essentially the same in both forms.

I will now pass on to a description of the bacillar forms which I have been able to investigate.

2. Bacillar Forms.

In two previous papers (Dobell, 1908, 1909), I have given a description of the structure and method of spore-formation in two large Bacteria which I obtained from the large intestines of frogs and toads. These two forms I named Bacillus flexilis and Bacillus spirogyra. The former is characterised by having a nucleus in the form of chromidia scattered through the cytoplasm: the latter by having a nucleus in the form of a spiral or zig-zag filament. B. flexilis, moreover, is a very large, flexible organism and forms two spores: whereas B. spirogyra is considerably smaller, rigid, and forms a single spore. As most of the Bacilli which I am now about to describe are organised in a manner similar to that of these forms, I shall—for convenience—refer to them frequently as Bacilli of the flexilis type or spirogyra type; meaning thereby that the organisms under discussion are structurally similar to one of these forms, though implying nothing as regards difference or identity of species.

(A) Bacilli of the flexilis Type.

(1) Bacillus flexilis.—Although I have already given a detailed account of this organism (Dobell, 1908), I shall here add a few further observations on its structure, as it seems to me of considerable importance that its cytology should be made absolutely certain.

My original figures were drawn from preparations stained by Giemsa's method. The various modifications of this method which I employed I have already given—as also several other methods which gave me satisfactory results. I would here emphasise the fact that all methods which give reliable cytological results reveal exactly the same structure in this organism. They show a number of deeply staining granules scattered through the cytoplasm—an appearance which I have interpreted as a nucleus in the form of chromidia. Subsequent work on this and allied forms has convinced me of the correctness of this interpretation.

On account of its very large size, B. flexilis is particularly well suited for observations upon its structure. I will now describe the appearances which it presents when fixed by a good wet method and stained by a good cytological stain.

I have given two figures of organisms so treated (Pl. 18, figs. 119, 120). Fixation, sublimate-alcohol (Schaudinn); stain, Heidenhain's iron-hæmatoxylin. It may be noted here that although this method gives good results on the whole, it is very difficult to obtain uniformly sharp differentiation. Different individuals behave differently towards the stain, so that in the same preparation well-stained, over-stained and under-stained organisms are often found side by side.

When examined under the highest magnification which I have been able to use (Zeiss 2 mm. apochromatic oil-immersion, compensating ocular 18) the following internal structure can be made out. The cytoplasm appears homogeneous and very finely granular (as it does in life) or else shows a rather indistinct alveolar arrangement (cf. fig. 120). The very well-marked cytoplasmic alveoli described by Schaudinn (1902) in B. bütschlii are very much more distinct than anything I have ever seen in B. flexilis. In the latter the cytoplasm is, at most, slightly alveolar. A number of round black granules can be seen scattered through each cell (figs. 119, 120). They are usually more numerous towards the periphery than in the centre. These granules constitute—I believe—the nucleus, and are probably composed largely of chromatin. Their behaviour during spore-formation I have already described (Dobell, 1908). No other structures are to be seen.

I believe that the figures (figs. 119, 120) give a faithful picture of the structure of B. flexilis. I am convinced that were other structures present—e. g. a vesicular nucleus, or a nuclear filament—they would have been visible in some of my preparations. I conclude, therefore, that the internal structure of B. flexilis consists simply of a faintly alveolar cytoplasm in which small granules of chromatin are imbedded.

Having said so much about B. flexilis itself, I will now

describe some very similar forms which I have been able to examine.

(2) Bacilli of the flexilis type from Lacerta muralis.—I found these organisms in the large intestine of Lacerta muralis captured in the neighbourhood of Naples. Many lizards which I examined contained these organisms, but I have no record of the exact percentage of animals infected. Possibly it is the same organism which Prowazek[1] observed in lizards in Rovigno. But from his very brief mention of it as "ein grosser mit zwei Polkörpern ausgestatteter Bazillus," it is impossible to be certain.

These bacilli are of very large size, but they are usually smaller than B. flexilis. The largest individuals which I have measured are about 30 μ in length, with a breadth of rather less than 2 μ. The ends are blunter than in B. flexilis, but the organisms are flexible in the same way. Figs. 83 and 84 (Pl. 17) show two of these forms after fixation with osmic vapour and staining with Giemsa. Figs. 133 and 134 (Pl. 18) show two other individuals—one of them (fig. 134) undergoing division—after fixation with sublimate-alcohol and staining with Heidenhain. It will be seen that all of them show essentially the same structure—a structure, moreover, identical with that of B. flexilis. There is a uniform or slightly alveolar cytoplasm containing chromatin granules scattered through it.

Division takes place in this organism by constriction—not by septation—as in B. flexilis. I have not observed its method of forming spores, but I believe that it is probably a disporic form.

In addition to this organism of a typical flexilis form, I found some smaller Bacteria, which show a similar kind of organisation, in the large intestine of L. muralis. They were of different sizes and forms, and may be different species or stages in the life of the same species. It is impossible to be certain from a comparison of the fixed and stained organ-

[1] "Untersuchungen über einige parasitische Flagellaten," 'Arb. kaiserl. Gesundheitsamte,' xxi. 1904, p. 1.

isms only. Some of these organisms are of large size, and it is easy to determine their structure. Two individuals of long, slender form are shown in figs. 64 and 65 (Pl. 17). They exhibit a pale, uniformly stained cytoplasm, with relatively large chromatin granules distributed through it. The cytoplasm is generally free from chromatin granules at the extreme ends of the organism.

This form divides like B. flexilis. It is slightly flexible, and motile. I have not observed spore-formation. The average length is about 11 μ, the breadth a little less than 1 μ.

I have seen a good many forms which are intermediate in size between these forms and the large flexilis forms described above in the same host. I think it possible that there may be some genetic connection between them, though it does not appear very probable.

(3) Bacilli of the flexilis type from Mabuia carinata.—In the large intestine of a Ceylon lizard, Mabuia carinata, I found an organism which is very similar to the flexilis forms from European frogs, toads, and lizards. The infected Mabuiæ were taken in Colombo.

This organism is shown in figs. 21–23, Pl. 16. It is motile, flexible, disporic (see fig. 23), and divides by constriction. It shows a nuclear apparatus exactly like that of B. flexilis. I have not been able to obtain all the stages in spore-formation, so that I do not know whether it displays the same remarkable phenomena during this process as are seen in B. bütschlii and B. flexilis. But such stages as I have found are very like those of B. flexilis. The average length of the organism is about 17 μ—a good deal less than that of B. flexilis or the flexilis forms from Lacerta muralis.

As far as my observations go, the ordinary forms of these organisms are therefore closely similar. There are differences which distinguish these three forms (i.e. those from the frog and toad [B. flexilis], from Lacerta, and from Mabuia) from one another, but cytologically they are all three essentially similar.

In the preparations of the contents of the large intestine of Mabuia, I also found some small Bacteria which show a similar structure. These forms (fig. 41, Pl. 16; fig. 76, Pl. 17) do not exceed about 10 μ in length, and are very slender. They all show a nucleus consisting of scattered granules of chromatin during the vegetative existence of the organism.

The Bacilli of the flexilis type from frogs, toads, Lacerta, and Mabuia all appear to have but one form of nucleus—that of a diffuse system of chromidia. But in addition to these forms, I have encountered two other organisms which —whilst apparently belonging to the same group—present certain features which separate them from the others. These two organisms were found in the common English newt and the Naples lizard, but to my great regret my observations—recorded in the ensuing pages—are very incomplete on both of them.

(4) Bacilli of a modified flexilis form from Triton vulgaris and Lacerta muralis.

(A) The form from Triton vulgaris.—I recorded in a previous paper (Dobell, 1908, p. 122) the fact that an organism similar to B. flexilis occurs in the newt. Unfortunately it is—as far as my observations go—exceedingly rare. I have found it only once, inhabiting the large intestine of a T. vulgaris captured in the vicinity of Cambridge. Subsequent search for the organism in other newts has up to the present been fruitless.

The organism is very long and slender. It is also the most flexible of all the flexilis forms which I have encountered. Many individuals attain a length of 30 μ and more, though the breadth is only about 1 μ.

The living organisms were actively motile. They showed a number of granules of various sizes in the cytoplasm, but no other clearly visible structures.

After fixation with formalin, and staining with Giemsa (see p. 415), the organisms could be seen to have the following structure (see figs. 79–82, Pl. 17). Most of the cells presented a nuclear apparatus like that of B. flexilis (fig. 79);

that is to say, they showed a number of red granules scattered irregularly through the cytoplasm. The latter showed no distinct structure as a rule.

Now, in addition to these forms of the characteristic flexilis type, there were other forms which possessed quite a different sort of nuclear arrangement (figs. 80, 82). They showed a variable number of large, nucleus-like bodies arranged in a single row along the whole length of the organism. The cytoplasm of these forms was finely granular (fig. 80), or sometimes distinctly alveolar (fig. 82). I observed a number of forms which appeared to be intermediate in structure between these forms and the ordinary chromidial forms; but I cannot state with absolute certainty that these different forms are not really different species. Though I believe that all the forms belong to one and the same species —representing different phases in the life-history—the fact that they are possibly different species living side by side cannot be ignored.

This organism is—like the other flexilis forms—disporic. It forms two terminal spores in each cell, but unfortunately I did not succeed in observing all the stages of spore formation. I have seen the living, actively motile, spore-bearing individuals, and also some isolated stages in the formation of the spores. Fig. 81 shows one of these—with two large chromatin spore-rudiments (as in B. flexilis) at the ends of the cell. I have not found stages in which the chromatin is arranged in a spiral filament—as in B. bütschlii and B. flexilis during spore-formation. It is possible, therefore, that spore-formation does not take place in the same manner as in B. flexilis, and that the forms which show large nuclei arranged in a row are really forms which are about to sporulate. This seems to me improbable, however, because both long and short individuals (figs. 80, 82) may show this arrangement of the chromatin, whilst it is usually in long individuals only that spores are found.

As far as my observations go, therefore, it appears probable that this particular organism, whilst presenting the flexible

form, disporic habit, and chromidial nucleus characteristic of other flexilis forms, has also stages in its life-cycle during which the chromatin is arranged in a series of large nucleus-like masses. The significance of this arrangement has not been determined.

(B) The form from Lacerta muralis.—I obtained this organism in a single lizard which I caught near Naples (Pozzuoli). As in the case of the preceding organism, I have been able to make only very fragmentary observations upon it. Unfortunately, I examined it only casually in the living state—believing it to be the ordinary flexilis form described above (p. 428). When alive it showed active movements and was flexible—like the ordinary flexilis forms from the lizard.

To my great regret I made only a single preparation of this organism—a dry smear on a coverslip, fixed in absolute alcohol and stained by Giemsa's method. All my observations, therefore, are based on this scanty and unsatisfactorily treated material.

It might be urged that the appearances presented by the organisms in this preparation are due to imperfect fixation. This may perhaps be true to some extent, but I do not think it is altogether justified. In the first place, other organisms in the same preparation (e.g. some spirogyra forms and other bacilli) appear quite normal. In the second place, drying and fixing in this manner never produces similar appearances in the ordinary flexilis forms.

Some of the organisms in this slide are shown in figs. 85–90 (Pl. 17). Many of them are of the form seen in fig. 89—that is to say, they present the appearance of long, slender Bacteria of the flexilis type. They possess a nucleus of the chromidial type—many of the chromatin granules being, however, relatively very large. Large individuals reach rather over 40 μ in length, though most of them are narrower than the ordinary flexilis forms (cf. figs. 89 and 83).

I have found in addition to these characteristic flexilis forms a number of individuals which display quite a different kind of nuclear arrangement. The chromatin is in large,

more or less spherical, nucleus-like masses (figs. 85, 88, 90). Some individuals possess three or four of these structures (fig. 85), whilst others—usually much shorter—possess but one (fig. 88). These "nuclei" bear a strong resemblance to the nucleus-like spore-rudiments of B. flexilis and similar forms, and it is possible that they are of a similar nature. Spores, however, are not present in any of the organisms.

A third modification of the chromatin which I found is that shown in figs. 86 and 87. It will be seen that the chromatic elements are neither in the form of diffuse chromidia, nor in the form of aggregated "nuclei," but in the form of a broken spiral filament. In no single case did I observe a complete filament like that of B. spirogyra. In the organism depicted in fig. 86 the chromatin is disposed as follows: a dumb-bell-shaped figure, two nucleus-like masses, and a short spiral filament with enlarged ends. Fig. 87 shows a single, nucleus-like mass, a short spiral filament, a small chromatin rodlet, a small dumb-bell figure, and a second more drawn out spiral filament.

After examining all these different forms of nuclear apparatus, my impression is that they are different stages in the life-history which are connected with one another in the following way. The chromidial form (fig. 89) becomes converted into the "nucleated" form (fig. 85) by the aggregation of chromatin at various centres; this nucleated form then gives rise to the form with the spiral filaments (fig. 87) owing to the drawing out of the nuclei by a process similar to that seen occasionally in the division of the nuclei in Micrococci (compare fig. 52). This is very strongly suggested in the organisms shown in figs. 86 and 87.

That this interpretation of the appearances is correct I cannot, of course, be certain. But the appearances are so suggestive that I cannot refrain from making the suggestion that in this organism we have a clue to much that is obscure in the morphology of the bacterial nucleus. It seems to me highly probable that in this organism three different arrangements of the chromatin—chromidia, spherical nucleus, and

spiral filament—succeed one another at different times in the life-cycle. But I will reserve a discussion of this for another part of the paper (see p. 481).

(B) Bacilli of the spirogyra Type.

Under this heading I will describe those Bacilli which possess a structure like that of *Bacillus spirogyra*. I have encountered many Bacteria of this form, and believe the type of organisation which they display to be a very common one.

(1) *Bacillus spirogyra*.—I have already given a full account (Dobell, 1909) of the structure and life-history of this *Bacillus*, which occurs in the large intestine of *Rana temporaria* and *Bufo vulgaris*. My published figures are all of organisms fixed with formalin and stained with Giemsa. As this method involves drying before mounting (see p. 415), I have thought it advisable to publish a few more figures of the organism after treatment by a more approved method, though—as I have pointed out on p. 416—drying after fixation with osmic acid or formalin does not appreciably affect the appearance of the cells as a rule.

Figs. 117 and 118 (Pl. 18) are accurate drawings of *Bacillus spirogyra* fixed with corrosive sublimate and acetic acid and stained with Heidenhain's iron-hæmatoxylin. No drying was allowed to take place at any stage during the making of the preparation—a moist film cover-glass preparation of the undiluted contents of the large intestine of a toad (*B. vulgaris*). It is very difficult to obtain good preparations of this organism with iron-hæmatoxylin, on account of the strong affinity of the pellicle for the stain. During differentiation the nuclear filament and the pellicle give up the stain at almost the same rate, so that one is apt to over-differentiate. But when differentiation has been stopped at exactly the right moment, very distinct figures of the cell-structure can be obtained.

The appearances presented by the cells after this method of treatment are essentially the same as those obtained by

osmic or formalin fixation and Giemsa staining. This can easily be seen by anyone who will compare figs. 117 and 118 with the figures which I have already published (Dobell, 1909). The cytoplasm has a uniform appearance—no granular or alveolar structure being apparent. In each cell a darkly staining filament (cf. fig. 117) can be seen. This filament usually has a more or less strongly marked spiral or zig-zag disposition, and extends throughout nearly the whole length of the cell. Its behaviour during cell-division and spore-formation I have already described in detail (Dobell, 1909).

In my previous papers I described this filament as a nucleus. As I now believe that this interpretation is completely justified from my subsequent observations,[1] I shall continue to refer to this structure by this name. I will say no more about the nucleus of B. spirogyra here, as I have already discussed it at some length in my previous papers, but I will now pass to a description of some other Bacteria which possess the same kind of structure.

(2) Bacilli of the spirogyra type from Lacerta muralis.—In my description of the cocco-bacillar forms which I found in the gut of Lacerta muralis, I pointed out (p. 422) that many cocci and cocco-bacilli showed nuclei of a more or less spiral form (cf. fig. 50, Pl. 17). Larger Bacteria of a more definite bacillar form show an even greater structural resemblance to B. spirogyra.

In the first place, there is a large Bacillus, which I found very frequently in the lizards whose rectal contents I examined, which is almost identical—as regards structure—with the B. spirogyra which I found in English frogs and toads. It is a large motile organism (figs. 55–60, Pl. 17), rod-shaped, with rounded ends, and reaches a length of 9 μ or slightly over. Dividing forms frequently exceed 10 μ in length.

The nucleus—as in B. spirogyra—is in the form of a filament of chromatin, arranged in a more or less spiral or zig-zag manner. It may be almost linear (fig. 55), thrown into a few coils (fig. 57), or much contorted (fig. 60). Sometimes

[1] See discussion on p. 466, et seq.

it has a much shortened and thickened form (fig. 56). It displays, in fact, the same range of variations which I have already described in B. spirogyra.

Division of the cell is preceded by division of the nucleus into two portions (cf. figs. 59, 60), exactly as in B. spirogyra. The organism is, moveover, monosporic—forming a single terminal spore in each cell (fig. 58). I have not followed out the whole of the stages in spore formation, but it appears closely similar to what I have already described in detail in the form from the frog and toad. (See Dobell, 1909.)

These figures (figs. 55–60) are all from preparations treated by the osmic-Giemsa method (p. 415). I have, however, obtained confirmatory results by other methods. In figs. 91–95 (Pl. 18) organisms are shown which have been fixed with sublimate-alcohol and stained with Heidenhain's iron hæmatoxylin. It will be seen that the structure of the cells is essentially the same as in the osmic-Giemsa preparations. This organism presents the same difficulties in staining by iron-hæmatoxylin as B. spirogyra (see p. 434). The individuals depicted are therefore selected specimens in which differentiation is particularly good.

As in the case of B. spirogyra, there can be no possible doubt of the existence of the spiral filament which I regard as the nucleus. Giemsa preparations reveal it always with the greatest distinctness, and Heidenhain staining—when successful—does the same. Though my interpretation may be wrong, the structures which I have described and figured exist beyond all shadow of doubt.

I found a large number of organisms of the same type which are intermediate in size between the forms just described and the cocco-bacillar forms described on p. 422. In fact I found such a gradually graded series of forms that it is difficult to me to avoid the conclusion that the smallest cocci and the largest bacilli are genetically connected. Direct proof of this most important point is wanting. Nevertheless, whether the different forms are different species or one and the same

does not in any way affect the fact that all the different forms exist. I have represented some of the small bacillar forms in fig. 51 (Pl. 17). They are so closely similar to the ordinary spirogyra forms, that it will be unnecessary to enter into a minute description of them. I would emphasise the fact, however, that many organisms exist which are intermediate in size and structure between these forms and the cocco-bacillar forms (fig. 50) on the one hand, and the long spirogyra forms (figs. 55–60) on the other. The importance of this fact will be discussed later (see p. 484, et seq.).

Amongst the smaller bacilli inhabiting the large intestine of L. muralis is a form which—though not of the characteristic spirogyra type—may conveniently be described here. The organism (figs. 53, 54, Pl. 17) is a short rod, about 3 μ in length. Each cell contains a nucleus in the form of a short, straight chromatin rod (fig. 53). This rod divides in the process of cell division (fig. 54), behaving like the spiral filament of ordinary spirogyra forms. This form is of interest because it is but a little removed from many of the cocco-bacillar forms. It may, indeed, be derived from a coccus-form by simple elongation. It is—structurally—a drawn-out coccus such as those in fig. 45, the nucleus assuming a straight rod-like shape instead of the spiral or zig-zag of many cocco-bacilli (cf. fig. 50). Some similar organisms from another lizard are shown in fig. 109 (Pl. 18). The drawings were made from a preparation fixed with sublimate alcohol and stained with Heidenhain. Inside each cell a short, darkly stained rod can be seen. I interpret this as the nucleus, though it may possibly be a spore-rudiment. In the case of the previously described individuals (figs. 53 and 54), however, such an explanation can hardly be advanced. For the deeply staining rods are present in forms which are undergoing division (fig. 54) and divide into two transversely during this process.

Another form of Bacillus which I found is one of large size (up to 8 μ in length) which has its nucleus constantly in the short, thick varicose condition which can sometimes be

seen in ordinary spirogyra forms (fig. 56). Some of these organisms are shown in fig. 63, Pl. 17. The nucleus is considerably shorter than the cell, and always has the thickened, knotted appearance shown in the figures; otherwise it resembles the nucleus of typical spirogyra forms.

(3) Bacilli of the spirogyra type from Mabuia carinata.—In these lizards, captured in Colombo, I found a large number of spirogyra forms in addition to the cocci and flexilis forms already described. All the preparations which I made of the contents of the large intestine were fixed with osmic acid and stained with Giemsa's or Leishman's stain. The following descriptions are therefore based upon material so treated.

The largest forms which I found (figs. 30–35, Pl. 16) are of considerable size. They reach a length of 16 μ. They show the organisation characteristic of spirogyra type organisms with great distinctness. Forms with a nearly straight nuclear filament (fig. 35), forms with a simple spiral or zig-zag (fig. 34) and forms with a very much twisted filament (fig. 31) were found. In fact all the modifications which are seen in B. spirogyra itself were encountered. A modification which I have observed comparatively rarely in B. spirogyra was fairly common in this form. In this modification the nuclear filament appears to be split at certain points, thus giving rise to open loops in these places (cf. fig. 32).

Division takes place as in B. spirogyra (fig. 33). It will be unnecessary therefore to describe it in detail. A number of short forms (fig. 36), which measured about 7 μ in length, were seen. They are apparently young forms resulting from the transverse divisions of the long individuals, but some of them may possibly be another species.

This organism is—like B. spirogyra and the spirogyra forms from Lacerta—monosporic. It forms a single terminal spore in each cell (see fig. 39). Details of spore-formation were not observed, but it appears to take place in a manner similar to that of B. spirogyra.

I found a few forms which appeared to belong to this organism, but which presented unusual features. I have figured two of these (figs. 37, 38, Pl. 16). The organism represented in fig. 37 has finely granular, darkly coloured cytoplasm, containing several large deeply stained chromatin masses. The organism shown in fig. 38 contains what appears to be a broken-up spiral filament together with some small chromatic granules. Whether these forms are normal stages in the life-cycle or degenerate forms, I am unable to decide. At first I took them for degeneration forms, but in view of the nuclear conditions which I have observed in B. saccobranchi (see p. 441), it seems possible that they represent normal events in the life-history. Their presence in my preparations seems worthy of record.

Another form, which I believe to be certainly a degenerate form, is that shown in fig. 40. I have seen and described (Dobell, 1909, Pl. 13, fig. 19) similar degenerate forms in B. spirogyra.

Many small Bacteria from the large intestine of Mabuia carinata showed nuclei of the spirogyra type. Drawings of some of these organisms will be found on Pl. 17. Fig. 69 shows a long slender form—11 μ long, and less than 1 μ broad. Through almost the entire length of the organism, a delicate chromatin filament—irregularly coiled and zig-zagged—can be seen to extend. In spite of its small size, the filament can be seen with the greatest clearness. Figs. 70, 71 and 73 show similar, but smaller organisms. In each of these the characteristic nuclear filament is plainly visible. Figs. 72 and 74 are smaller forms (length of fig. 72 = 2 μ) with relatively short, thick and straight nuclear filaments. The nucleus appears to be like that of the organism shown in fig. 56, but on a very small scale. Fig. 75 shows a dividing form of the same kind of organism as fig. 72. With the exception of certain forms from the blood of Saccobranchus (see p. 441), these are the smallest Bacilli in which I have succeeded in differentiating a nuclear filament with absolute precision. All the figures are faithful copies of the organisms

which were in my preparations. Their structure was as distinct as that of much larger forms.

Two other organisms are shown in figs. 77 and 78. In the form shown in fig. 77, there is apparently a large nuclear mass occupying most of the middle region of the cell. I found several organisms like this one. They were all equally distinct, and appeared normal. But it is possible they are really plasmolysed forms of one of the other varieties. I cannot be absolutely certain that they are not, as I have not sufficient material for comparison. Fig. 78 is drawn from an organism which has its nucleus in the form of a slightly bent rod. I found a fair number of individuals of this form. It appears to be a large form similar to the Lacerta organisms shown in fig. 53. It is also somewhat similar to the forms shown in fig. 63.

I believe that a number of very small Bacteria which occur in frogs, toads, and lizards have the same structure as these small Bacteria which I have just described. But I have, up to the present, been unable to convince myself that this is so. With very small Bacilli it is usually very difficult to be absolutely certain of the exact structure. Only in very favourable cases, when staining has been exactly right—as in these minute forms from Mabuia—can satisfactory results be attained.

(4) Bacilli of the spirogyra type from Bufo melanostictus.—In the large intestine of this toad—taken near Colombo—I found several Bacilli which showed the spirogyra type of nuclear structure. I have given figures of two different forms which I encountered (see Pl. 17, figs. 61, 62).

The larger forms (fig. 62) were usually of the shape of a bent rod. They attained a length of 10μ. In these organisms the nuclear filament was usually linear rather than twisted—like the straight form of filament in B. spirogyra. It may be described as a slender thread with here and there large knot-like swellings on it (fig. 62). I did not see any individuals with definite spiral or zig-zag filaments.

The smaller forms measured about $2\,\mu$–$2{\cdot}5\,\mu$ in length. They were of short, oblong form (fig. 61), and each contained a very distinct spiral or S-shaped nuclear filament. I give pictures of three individuals of this form in fig. 61, and will not describe them in greater detail.

(c) Bacillus saccobranchi n. sp. and Associated Forms.

I shall now describe a new Bacillus which shows a form of nuclear apparatus differing in many ways from typical flexilis or spirogyra organisms. As it is convenient to have a name for this organism, I propose to call it Bacillus saccobranchi.

(1) B. saccobranchi n. sp.—I obtained this Bacillus in the following manner. Whilst I was working at the Colombo Museum, I examined the blood of a number of fish for trypanosomes. Among these I examined a number of individuals of Saccobranchus fossilis caught in the Colombo Lake. Some of them contained T. saccobranchi Cast. et Will. in their blood, but the majority showed no blood-parasites of any sort.

One day two Saccobranchi were brought to me for examination. As I was unable at the moment to examine their blood microscopically, I placed them in water in a large earthenware chatty. On the following day, when I was about to make an examination of the blood of these two fish, I found that one of them had died during the night. As it appeared quite fresh, however, I decided to examine its blood for Protozoa. The fresh blood, taken from the heart, showed no Protozoa, but contained some very large and actively motile bacilli, together with a number of smaller Bacteria.[1] As the bacilli seemed to me particularly suitable for study—both on account of their size and the medium in

[1] I never found Bacteria in the blood of other fish which I killed and examined immediately after killing. It is possible that these Bacilli caused the death of this particular fish, though there is no conclusive evidence that this is so.

which they were living—I made some moist films in the following way. The blood was spread in a thin film upon a slide and immediately—without drying—exposed to osmic vapour for about 30 seconds. The slide was then transferred immediately to absolute alcohol, where it was left to harden for 15 minutes. After this treatment the film was stained in the usual way in Giemsa's stain—finally being allowed to dry. I made several preparations in this way, and also by the ordinary drying method with fixation in absolute alcohol. In the preparations fixed with osmic vapour, both the blood-corpuscles and Bacteria were beautifully preserved, and the description of the organisms which follows is taken from these preparations.

The largest individuals of this form are of considerable size (figs. 4–6, Pl. 16). They attain a length of as much as 16 μ, and a breadth of 2 μ. The cytoplasm contains no inclusions and appears usually homogeneous, though occasionally it has a slightly alveolar structure. In all the Giemsa preparations the cytoplasm is coloured a bright blue (see figs. 1–20)—as I did not extract the colour with alcohol after staining.

In all the organisms the chromatin is stained red—in sharp contrast with the cytoplasm (see figs. 1–20). It is distributed through the cell in various ways, which can be classified conveniently into three main types of structure, with intermediate conditions.

The first type of nuclear structure is a typical spirogyra form (figs. 1, 2, 4, 12). The nucleus is in the form of a spiral or zig-zag filament, extending through the greater part of the length of the cell. As in B. spirogyra, the filament may be comparatively straight (fig. 1) or much contorted (fig. 2). All intermediate conditions occur. During division the filament behaves in the same way as in B. spirogyra: that is to say, it divides transversely into two, a half of the original filament remaining in each daughter-cell (see fig. 2).

The second type of nuclear structure is that shown in figs. 3, 5–9. The organisms of this type show nuclei of very irregular form. They possess chromatin structures in the

form of short, irregular, broken, bent, branched, and twisted filaments (cf. figs. 3, 6, etc.). These filaments are sometimes connected with one another to form irregular networks (fig. 9). In addition to the filamentar chromatin bodies, granules and irregular masses of various shapes and sizes are frequently present (figs. 3, 5, etc.). This irregular form of nucleus appears to be derived from the spirogyra form through the breaking up and rearrangement of the elements composing the spiral filament. Figs. 6 and 8 show organisms which suggest this very clearly. Indeed, I have seen so many forms intermediate between these forms and the typical spirogyra forms that I have no doubt at all that they are different nuclear stages which succeed one another at different periods in the life-history.

The third type of nuclear structure is the chromidial type, characteristic of the flexilis group of organisms (fig. 10). In the organisms of this type—which were present in comparatively small numbers in my preparations—all the chromatin is in the form of minute, diffusely arranged granules (fig. 10). It can generally be seen that the chromatin structures (filaments, networks, etc.) of the other forms are built up of aggregated granules of chromatin. The organisms with a chromidial type of nucleus appear to be derived from the forms with irregular nuclei by the breaking apart of these chromatin granules. In a form such as that shown in fig. 5, the irregular chromatin filaments and masses appear to be breaking up. Other organisms show later stages in this process—intermediate between figs. 5 and 10. I believe, therefore, that the chromidial type is derived from the irregular type through the dispersal of the chromatin granules.

As a result of examining a considerable number of individuals, therefore, I have come to the following conclusions: B. saccobranchi may possess a nucleus of the characteristic spirogyra type, or of the characteristic flexilis type. It shows in addition a large number of forms intermediate between these two types. The nucleus in the intermediate

forms may have the form of a broken filament, of an irregular arrangement of branched filaments or chromatin masses, or of an irregular network. So many different forms exist that it is difficult to classify them accurately.

On account of the large number of intermediate forms, it seems to me certain that all these different organisms are not really different species. They grade into one another almost imperceptibly, so that I regard the interpretation of them as different species as excluded. Whether the spirogyra type gives rise to the irregular type, and this in turn to the flexilis type, or whether the process is in the reverse order, cannot, of course, be stated with absolute certainty from an examination of fixed and stained material alone. The former interpretation, however, appears to me the more probable. It is really immaterial which of these interpretations is correct. The point of interest is that all these different nuclear modifications occur in the same organisms. I shall discuss the matter more fully later (see p. 471).

This organism is, like typical spirogyra forms, monosporic. The spore is formed towards—but not absolutely at—one end of the cell (see fig. 14). I have not found all the stages in spore-formation in my preparations, but such stages as I have seen are similar to those of B. spirogyra. As in this form also, the spore-forming individuals are short—measuring on an average about 8 μ in length. A certain amount of chromatin, also, is left over in the formation of the spore (fig. 14).

(2) Forms associated with B. saccobranchi.—I have already noted above (p. 441) that I found a number of smaller Bacteria in my preparations of the blood of Saccobranchus. These forms I will now describe.

First of all, I should point out that although I found many very small individuals, which were easily distinguished from the large forms which I have called B. saccobranchi, yet many of the smaller forms were really but little smaller than this form. Indeed, I found so many organisms of all sizes intermediate between the largest B. saccobranchi and the

most minute Bacilli, that I cannot avoid the impression that all these different forms are really connected with one another. This would be a point of great interest if it could be established, but unfortunately no definite conclusion can be reached by simply examining dead organisms.[1]

I found a number of individuals—of smaller size than the large bacilli—which showed a very distinct nucleus of the spirogyra type. Some of these are shown in figs. 11 and 15. They measure about 6 μ in length, and are considerably narrower than the large organisms which I call B. saccobranchi. Each individual will be seen to contain a characteristic nuclear filament of the spirogyra type.

Some smaller forms, with a similar structure, are shown in fig. 20. They measure about 3 μ in length, and are much narrower than the preceding forms: otherwise they are closely similar. Nuclear filaments of various forms are seen in each cell.

Some still smaller organisms—2 μ and less in length—are depicted in fig. 19. In spite of their very minute size, they show a nuclear filament with great distinctness. They appear to possess a nucleus exactly like that of the spirogyra forms of B. saccobranchi (fig. 2, etc.), but on a very small scale. These are the smallest bacilli which I found in the blood of Saccobranchus.

I would again emphasise the fact that the various individuals depicted in figs. 2, 11, 20, and 19 are organisms selected on account of the difference in their sizes. Actually, a number of intermediate-sized organisms of precisely similar form were seen.

I have added some figures of other organisms which were of interest. The Bacillus shown in fig. 13 shows a nucleus of a form which I observed in a few individuals. It is in the

[1] If the organisms are all of one species, it follows that the name B. saccobranchi should be given to all. As the matter is so doubtful, however, I have restricted this name to the large forms only, though I do not wish to imply thereby that the large forms are specifically distinct.

form of a short, irregular mass of chromatin, which appears sometimes to be really a spiral filament contracted upon itself. Fig. 17 shows a pair of Bacilli with rod-like nuclei. Fig. 18 appears to be a similar kind of organism undergoing division. Fig. 16 is an individual which is like a B. saccobranchus, with a nucleus of the irregular type, on a smaller scale. None of the smaller forms showed nuclei of a distinct chromidial type.

3. Spirillar Forms.

I have endeavoured to elucidate the structure of a number of spirillar forms, but have succeeded in reaching definite results in only four different forms. Yet these four different forms have shown me three different types of nuclear apparatus, and these I shall now describe.

(A) Spirilla with Nuclei of a Chromidial Type.

In a previous paper (Dobell, 1908) I described briefly the structure and method of spore-formation which is found in the Spirillum—which I named S. monospora—which occurs very commonly in the large intestine of frogs and toads. I described this organism as containing a number of scattered granules, stainable with chromatin stains. These granules I regarded as constituting a nucleus of the same sort as that of B. flexilis and similar forms. They enter into the formation of the spore, forming a nucleus-like spore rudiment—as in B. flexilis, B. spirogyra, B. lunula, and many other Bacteria.

I have made many attempts to obtain a more detailed knowledge of S. monospora. My efforts, however, have been unavailing. The facts—as far as they go—which I have already recorded are the only ones which I have so far been able to discover. I therefore abandoned this form and looked for other and more favourable objects for investigation.

One of the first organisms to which I then turned my attention was the large Spirillum which inhabits the hind gut of the common cockroach, Stylopyga orientalis. I hoped—from the large size of this organism, and from the descriptions which have been given of it by others[1]—that I should find some new and illuminating structures in this Spirillum. But my hopes have not been altogether fulfilled.

The only structures which I have been able to make out in this form with absolute precision are essentially the same as in the form from frogs and toads. The Spirillum of the cockroach possesses an exceedingly thick cell wall, which has a strong affinity for many stains, and which renders it very difficult to study cytologically. I have obtained the most satisfactory results with wet films fixed in sublimate-alcohol and stained with iron-hæmatoxylin. But differentiation of the contents of the cell is very difficult to obtain, and succeeds only occasionally. It has usually been my experience that

[1] According to Guilliermond (1907) and others, an organism containing a typical nucleus has been described by Kunstler and Gineste ('C. R. Assoc. Anatomistes,' 1904), under the name Spirillum periplaneticum, from the intestine of Periplaneta orientalis. Unfortunately I have not been able to see this paper. In two subsequent notes, however (Kunstler and Gineste, 1906, 1906A) these authors describe and figure this organism, but do not mention or depict a nucleus, and give the host as P. americana. In the first note (1906) a reticular structure of the cytoplasm is figured and described. In the second note (1906A) "spherular elements" are described in the cytoplasm, and the average dimensions are given as $8\mu \times 3\mu$. It is possible that this is the same organism which I call the "large Spirillum from St. orientalis," but I cannot be certain from the incomplete notes of Kunstler and Gineste which are at my disposal. Fantham ('Quart. Journ. Micros. Sci.,' 1908) casually describes "spirillar forms occurring in the hind gut of the cockroach." He observed "a diffuse nucleus . . . consisting of a number of chromatin masses seen to be connected by a lightly staining spiral in successfully stained specimens. At other times the achromatic thread was not evident." Diagrammatic figures are given. They are not like anything I have seen in Spirilla from S. orientalis. I do not attach any importance to these fragmentary observations.

when the colour has been sufficiently extracted from the cell wall to enable one to see the internal structures, then the cell contents have also been so much decolorised as to be almost invisible. Now and then, however, clear pictures of the protoplasmic structures can be obtained.

In well-stained individuals I have invariably found the same structure present. There is no vesicular nucleus visible and no nuclear filament. The only structures which can be seen are shown in figs. 110–112 (Pl. 18). The cytoplasm is alveolar, the alveoli sometimes extending across the whole width of the cell, sometimes being of smaller size. At various points in the walls of the alveoli lie deeply staining granules. They are usually few in number, very irregularly arranged, but generally in close proximity to the cell wall. Occasionally two or more granules appear at first sight to be connected with one another by fine intervening filaments; but it seems to me that in all cases these filaments are really nothing more than the alveolar walls. I have never found any structure at all comparable with the nuclear filament of B. spirogyra and similar forms.

My interpretation of the appearances observed in the large Spirillum of the cockroach is therefore this: The structure is essentially the same as that of B. flexilis and allied organisms. The cytoplasm is alveolar and the nucleus is in the form of chromidia—consisting of a few small, scattered granules of chromatin lying in the walls of the alveoli. Neither vesicular nucleus nor nuclear filament exists in the forms which I have examined. The structure is therefore closely similar to that of S. monospora.

(B) Spirilla with Nuclei of Filamentar Form.

There is a large Spirillum[1] which lives in the gut of Lacerta muralis. I have found it much easier to study than the large forms from the frog and the cockroach, and

[1] These are the organisms to which I referred in a previous paper (Dobell, 1909, p. 583).

have found that it possesses a different kind of organisation. It is—like the other forms—difficult to stain satisfactorily, but I have obtained some beautiful results with iron-hæmatoxylin. My figures (figs. 96–108, Pl. 18) are all drawn from wet film preparations, fixed with sublimate alcohol and stained with Heidenhain's iron-hæmatoxylin. My best preparations are from the contents of the large intestine of lizards which I obtained from a dealer in Munich; but similar—I believe the same—Spirilla also occur in Naples lizards, and have furnished me with additional material.

The structure of these Spirilla is shown in figs. 96–108. Long individuals attain a length of as much as 13 μ (fig. 102) and more, whilst the shortest individuals measure about 4 μ (figs. 103–105). All forms display a structure which is essentially the same, and which differs markedly from that of other forms which I have investigated.

In fixed and stained organisms[1] the cytoplasm has, as a rule, a very distinct alveolar structure (cf. figs. 96, 100, 102, etc.). The alveoli may extend right across the cell, being arranged as a single row of large chambers (fig. 96), or they may be of variable smaller sizes (e. g. fig. 107). The only other structure which is normally present in well-stained individuals is a darkly staining filament, like that of B. spirogyra, which I interpret in this case also as the nucleus (see figs. 96–108). Both the form and position of this filament may vary. It is frequently in the form of a short and nearly straight rod (figs. 96, 104, etc.), occupying a central (figs. 104, 107) or terminal (fig. 96) position, or any intermediate site in the cell. It may be in the form of a more or less twisted and varicose spiral or zig-zag (figs. 101, 102, etc.), sometimes showing knob-like thickenings at the ends (figs. 100, 105), and sometimes running almost from one extremity of the cell to the other (figs. 100, 101, etc.). Inspec-

[1] As in the case of other Spirilla I have been unable to reach any definite conclusions regarding structure from a study of the living organisms. The living organisms, however, have been studied in all cases.

tion of the figures on Pl. 18 will give a better idea of the various modifications which occur in this filament than pages of description.

The filament is a morphological element present in each cell. It is a body which is independent of the walls of the cytoplasmic alveoli: that is to say, it is not merely an accidental appearance due to portions of the alveolar walls taking up the stain more strongly than others. It can be seen in many cases that the filament crosses the alveoli, and is a quite independent structure (cf. figs. 96, 102, etc.). I believe there can be no doubt whatsoever that it is a structure of precisely the same sort as occurs in B. spirogyra and so many other similar organisms.

Additional evidence in support of this view is derived from a study of the behaviour of this filament during cell division. Just as in the Bacilli of the spirogyra type, division of the filament precedes division of the cell as a whole (see fig. 108). After the filament has divided into two approximately equal parts, the cell divides across the middle (figs. 97, 98). It will be noted that during division the filament must lie in a central position in the cell, and that immediately after division it must occupy a more terminal position in the daughter-cells. This no doubt to a large extent explains the variable position of the filament, in different cells, alluded to above. On purely morphological grounds, therefore, it seems to me justifiable to regard the filament in these Spirilla as a nucleus of the same sort as occurs in Bacilli of the spirogyra type. I shall discuss these filaments and their interpretation later (see p. 466): for the present I wish simply to establish the fact that a filament of this sort is present in these Spirilla and in many Bacilli.

Occasionally darkly staining granules are present in the cytoplasm of the Spirilla from the lizard. They are few in number, and not a constant morphological feature of the cell (cf. figs. 101, 102). I regard them as probably composed of reserve material of some sort. As a rule these organisms are free from granular inclusions.

(c) Spirilla with Spherical Nuclei.

The fourth and last spirillar form concerning whose structure I have reached definite conclusions is exceedingly minute. It is an organism which occurs commonly in the hind gut of the common cockroach (S. orientalis) in company with the larger form already described.

The organism in question has the characteristic form of a small Spirillum or Vibrio (figs. 121–132, Pl. 18). The smallest Vibrio forms have a length of rather less than 2μ (fig. 132) and upwards. In the largest Spirillum forms the length rarely exceeds $4{\cdot}5\ \mu$ (fig. 130). In all cases the organisms are very narrow—the largest having a breadth of approximately $0{\cdot}5\ \mu$, the smallest, considerably less. Yet, in spite of their very small size, these organisms possess an internal structure which may be demonstrated with the greatest clearness.

I have obtained very good results with wet films, fixed with sublimate-alcohol and stained with Heidenhain. All the figures (figs. 121–132) on Pl. 18 are from individuals treated in this manner. Differentiation may be obtained with comparative ease.

Every individual—when properly stained—shows a single, darkly staining, spherical body situated somewhere within it (cf. figs. 121–124, 130–132). I regard this body as a nucleus. This body—or, as I shall call it, this nucleus, may lie in the centre of the cell (fig. 123, etc.) or at one extremity (fig. 121), or in an intermediate position. It always has the form of a minute round or oval granule, which appears—under the highest magnification which has been available—quite homogeneous. It is a constant morphological feature of every cell. No cytoplasmic structure can be made out with absolute certainty, on account of the very small size of most of the organisms. Faint indications of an alveolar structure seem to me, however, to be sometimes present. In addition, it may be noted that the ends of the cells frequently stain deeply, so that in optical section an individual often appears

to have its ends covered with little dark caps—an appearance seen in many stained Bacteria.

During the division of an organism into two, the nucleus behaves in a characteristic manner. It lies, before division, towards the centre of the cell (fig. 130). Whilst in this position, it divides into two daughter-nuclei, with the formation of a minute dumb-bell figure (figs. 125, 126). These daughter-nuclei then separate to a variable distance from one another (fig. 127) and cytoplasmic fission follows (figs. 128, 129). According to the degree of separation which has occurred between the daughter-nuclei, the nuclei of the daughter-cells may lie in a terminal (fig. 129) or central (fig. 128) position.

The general appearance of the nucleus in this Spirillum —both during rest, and during division—is therefore closely similar to that of the nucleus which I have already described in Micrococci and Sarcina.

4. "Fusiform Bacteria."

At various times, I have encountered a number of organisms belonging to the group of so-called "fusiform Bacteria." These organisms are very commonly found in the intestines of animals. I have studied their structure in quite a number of forms, and all appear to be organised in essentially the same manner.

My own impression—after observing many different organisms of this class—is that they are really not Bacteria at all, but more probably belong to the Fungi. Perhaps they are related to the yeasts. But as I have no conclusive evidence —from their life-histories—of their real affinities, and as they are usually regarded as Bacteria, I will describe here the forms which I have investigated.

(A) "Fusiform Bacteria" from Lacerta muralis.

I found fusiform organisms fairly common in the large intestines of the lizard which I captured near Naples. They were

generally to be found in my preparations, but usually in small numbers.

Good preparations can be obtained by the osmic acid or formalin method with Giemsa staining (see p. 415), and Heidenhain's iron-hæmatoxylin—when sharp differentiation has been achieved—gives very good pictures of the structure of these organisms.

In Pl. 18, figs. 113 and 114, will be seen some fusiform organisms from a moist film preparation of the contents of the large intestine of L. muralis, fixed with sublimate-alcohol and stained with Heidenhain. It will be seen that each cell contains a single, darkly staining, centrally situated, spherical mass, which I believe to be the nucleus. I have not obtained any satisfactory preparations which show a division of this body in this particular form, but from analogy with similar organisms, I have little doubt that it divides in the course of cell division.

Small single individuals (fig. 114) have the characteristic spindle shape, and measure $4\,\mu - 4{\cdot}5\,\mu$ in length. The ends are sharply pointed. No other structures besides the nucleus were visible in the cells.

In this form—as in all the other fusiform organisms which I have investigated—double individuals are very common (fig. 113). They arise from dividing single individuals through an incomplete separation of the daughter-cells—in the same way that Diplococcus forms are produced by Micrococci.

Smaller forms than those just described also occur in the lizard. They all show a similar structure. Fig. 68, Pl. 17 is of a small double form, stained with Giemsa's stain. Each individual shows a distinct nucleus, as in the Heidenhain-stained individuals. The length of this double organism was approximately $5\,\mu$.

(B) "Fusiform Bacteria" from Frogs and Toads.

The common English frog and toad (R. temporaria and B. vulgaris) occasionally contain fusiform organisms in

their large intestines. They appear to be the same in both hosts, but I have found them more often in the toad than in the frog. Many of them, however, are of very small size, so that they are easily overlooked unless one is specially on the look-out for them.

As a rule these organisms occur in the double form (fig. 115)—single spindles being comparatively rare in any preparations. The ends of the double spindles are more rounded than in the forms from the lizard. The nuclei are of the same type. Fig. 115 is an individual (double) from the large intestine of Bufo vulgaris (moist film, sublimate-alcohol and Heidenhain). Heidenhain and Giemsa reveal the same structure. The length of the double individual depicted in this figure was ca. 10 μ.

(c) "Fusiform Bacteria" from Triton vulgaris.

The common newt contains several different forms of fusiform organism. They are similar to those of the frog and lizard, and usually occur in the double form. I have not made a very careful study of these organisms, but they show certain features of interest.

Each individual (figs. 66, 67, Pl. 17), contains a single nucleus. This may take the form of a simple, apparently solid granule of chromatin (cf. fig. 67, upper nucleus), or occasionally it appears to be a vesicular structure with one large karyosome (cf. fig. 67, lower nucleus). From the small size of these nuclei it is often very difficult to be quite certain of their exact structure. Another type of nucleus, which I observed in some of the larger forms from the newt, is that shown in fig. 66. This figure shows an ordinary double form in which the upper individual is dividing into two. It will be seen that all three nuclei which are present are in the form of double granules, arranged transversely across the cells. All these forms were seen in Giemsa preparations, which were fixed with formalin, and dried before mounting. It is therefore not impossible that

these double nuclei are artifacts produced by the breaking into two of a single granule through drying. This seems to me improbable, however.

(D) "Fusiform Bacteria" from Stylopyga orientalis.

The common cockroach harbours a fusiform organism very like those which I have already described. The forms which I have encountered are usually of small size, and are generally of the double spindle form.

Every cell contains a nucleus (see fig. 116). It is frequently of a rather square shape, and sometimes I have found nuclei which appear to be dividing (cf. lower nucleus in fig. 116). All the dividing nuclei which I have seen are of this rather curious form—that of two chromatin masses separated by a variable distance. I have not been able to make out any other details of the process of nuclear division, but I have not made a very careful study of these organisms. My only knowledge of them is derived from occasional individuals which I have found in preparations made for other organisms.

5. On some Nucleated Bacterium-like Organisms.

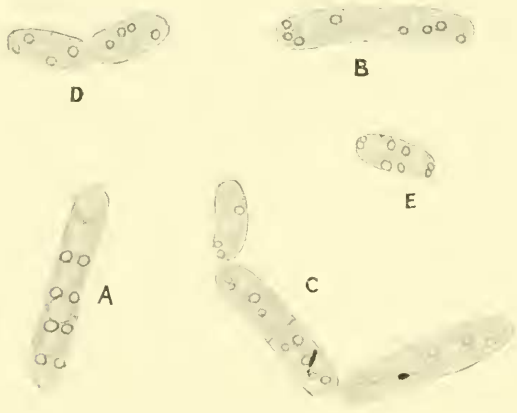
In this section I shall describe some curious organisms which present—at certain stages in their lives—a strong resemblance to Bacteria, but which are really to be placed among the Fungi. For a considerable time I believed these organisms to be Bacteria which possessed a typical vesicular nucleus—like that of Bacterium gammari—and I believe that other observers may have fallen into a similar error. I think, therefore, that no excuse is needed for publishing my results in some detail in the present paper.

The organisms under consideration have all been found in the large intestines of animals. I have found them in several insects, snakes, lizards, and Amphibia, and I have also encountered forms which I believe to be of a similar nature in the rectal contents of mammals. However, I have made no careful

investigation of these. The forms which I have studied most carefully are those occurring in frogs and toads (Rana temporaria and Bufo vulgaris), in the common cockroach (Stylopyga orientalis), and in Boa constrictor. It is upon the organisms from the large intestine of this snake that I have made the most complete series of observations, and I will therefore record them in some detail at this point.

The living organisms, when removed from the host, and examined under an immersion, appear as large, bacterium-like,

TEXT-FIG.



Bacterium-like organisms from large intestine of Boa constrictor. Living organisms. (Zeiss, 2·5 mm. apochromatic water immersion × compens.-oc. 18.)

non-motile rods (see Text-fig.). They may occur singly, in pairs, or in chains. The average length of the largest individuals is about 14 μ. Smaller individuals are very common, and many do not exceed 4 μ. All intermediate sizes are to be found.

The rods all have rounded ends, and many of the longer individuals show a slight curvature (cf. Text-fig. B). The internal structure in the living cells is very easily seen, though the nucleus can be satisfactorily demonstrated in stained preparations only. The cytoplasm is finely granular, and contains as a rule a number of refractive bodies—probably reserve

material. In addition to these, pale vacuoles are usually to be seen. They may be irregularly scattered through the cytoplasm (B, C) or arranged in a single line down the middle of the organism (A). In the latter case the refractive bodies frequently occur in pairs between the vacuoles—as shown in fig. A. Intra-vitam staining[1] brings out the refractive bodies very sharply, but does not reveal the nucleus.

Multiplication can be easily observed under the microscope. It is accomplished by the rods undergoing a transverse fission in a manner which closely resembles that of many Bacteria. If long cells be carefully scrutinised, some of them can be found which show faint indications of a septum towards the middle of the organism. The septum makes its first appearance as two faint transverse lines, extending towards one another from opposite sides, in the centre of the cell. A little later the lines appear to meet, so that a delicate septum extends right across the middle of the cell (see Text-fig. C —middle individual). The septum becomes thicker, and cuts the parent cell into two equal daughter-cells. After separation—which now takes place—the contiguous ends of the daughter-cells are square, but they rapidly assume a rounded appearance (cf. text-fig. D). The whole process of division —as seen under the microscope—takes several hours, proceeding very slowly. I have not been able to follow the division of the nucleus satisfactorily in the living organisms.

All the material upon which these observations are based came from the large intestine of a single B. constrictor which had been in captivity for some time. I have, therefore, no data to indicate the frequency with which the parasite occurs in this snake.

On examining the contents of the large intestine of the snake, soon after death, I found a large number of organisms present in the stages which I have just described. My first conclusion—not unnaturally—was that I was dealing with a large species of Bacterium. Had I not made further observations upon the subsequent development of the organ-

[1] With neutral red, methylene blue or Brillantcresylblau.

isms, this conclusion might have appeared to some extent justified. After fixing and staining some of the cells, a large nucleus was seen to be present. It therefore appeared to me at the time that I had discovered a new Bacterium which possessed a well-marked nucleus, and hence belonged to the group of organisms of which B. gammari is the type.

Stained examples of this organism from the boa are shown in figs. 137, 138, 140, 141 and 144 (Pl. 19) and in fig. 135 (Pl. 18). Owing to the watery nature of the rectal contents, and to the large amount of grit present, it was found very difficult to obtain good wet-film preparations. Most of my stained preparations were therefore made by allowing some of the fluid containing the organisms to dry upon a slide;[1] then fixing the dried film in absolute alcohol; and finally staining with Giemsa's stain. As a cytological method this is of course unsatisfactory; but the results obtained were, in the main, good enough for arriving at conclusions regarding the general structure of the cells. In most cases the nucleus had undergone a certain amount of fragmentation—owing to drying—but it frequently showed its vesicular structure quite clearly.

In fig. 137 a number of small individuals are depicted—each showing a distinct nucleus. Fig. 138 shows two larger individuals, of the characteristic Bacterium form, with rounded ends. Fig. 141 shows a similar organism, but with ends of a squarer form. The nucleus is in all cases unmistakable. In fig. 140 a chain consisting of four organisms of a more or less bent form is seen. The nuclei are all somewhat broken up through drying. Fig. 144 shows another chain of four individuals, of smaller size, and each containing a vesicular nucleus. Forms intermediate in size between these small forms and the larger forms occur, so that there is no reason for regarding them as different species. I propose to call all the individuals which have the rod-form characteristic

[1] At the time when these observations were made (1906) I had not discovered the osmic acid drop method of fixation which has since proved so useful.

of Bacteria, the bacterioid forms—to distinguish them from other forms.

The other forms which this organism is able to assume appeared in the course of a few days in the contents of the snake's large intestine, which had been kept as a culture in a glass vessel. They were not found inhabiting the snake. Multiplication of the bacterioid forms continued for several days, after which the other forms made their appearance. The ordinary bacterioid individuals (such as fig. 138, etc.) were seen to become more rounded (fig. 142), finally assuming the oval form characteristic of a yeast. In this yeast-like condition the organisms continued to multiply—but by budding, and not by transverse fission (see fig. 145). I propose to call these yeast-like forms the zymoid forms—to distinguish them from the rod-like bacterioid forms.

The zymoid forms are exactly like any other ordinary yeast. They possess an oval form, a vesicular nucleus, and reproduce by budding (cf. figs. 136 [Pl. 18] and 145 [Pl. 19]). They are, indeed, exactly like other yeasts with which I am familiar in the rectal contents of frogs, toads, lizards and many other animals.

That the zymoid forms are directly derived from the bacterioid forms—and are not really independent organisms—I can assert with absolute certainty. I have observed the transformation in living organisms kept under observation for several days. All intermediate forms, moreover, were found in my fixed and stained preparations, and it was no uncommon thing to observe bacterioid and zymoid individuals composing one and the same chain (fig. 151). Both bacterioid and zymoid forms existed side by side in my cultures for many days, but finally the bacterioid forms were almost completely supplanted by the zymoid forms.

Curious further changes were also observed. Many of the bacterioid forms developed outgrowths, which sometimes grew to a considerable length (see fig. 148). Many of the zymoid forms also gave rise to outgrowths—in some cases of very large size. These outgrowths began as short finger-like

processes (fig. 146), into which the nucleus sometimes entered (fig. 149). In some cases, division of both nucleus and cytoplasm occurred—the finger-like outgrowth being separated off as a more or less bacterioid cell (fig. 150). At other times, the outgrowths continued to grow in length without cell division taking place. They often attained a considerable length, and underwent branching (fig. 147)—looking like the beginnings of a mycelium. Although I kept the organisms under observation for many weeks, I never found any other stages in development. Apparently, the conditions under which the organisms were kept were such as to inhibit further growth.

Now after observing the changes which my original Bacterium-like organisms underwent, I came to the conclusion that I was really dealing with a fungus closely allied to the yeasts. It seems to me more than probable that the organisms are really fungi, a part only of whose life-cycle has come under my notice. I believe the resemblance of the original bacterioid forms to Bacteria is purely accidental, and the organisms have nothing whatever to do with this group.

As I have already noted, forms similar to these from Boa constrictor occur in the intestines of a variety of animals. It is therefore necessary to be on one's guard when investigating Bacteria derived from such sources. Unless observations be made upon the development of the living organisms, one may easily be led into error.

I must point out that the finer details of nuclear division —of both bacterioid and zymoid forms—have not been thoroughly investigated. This is due to the fact that perfect fixation was usually impossible. Division is, I believe, amitotic: and this is certainly true of the form which occurs in the frog—a form upon which I have made a number of careful observations. As these, however, are still incomplete, and indicate that this form is very closely similar to that from the boa, I do not wish to enter into a fuller description at present.

In conclusion, I would emphasise the fact that the foregoing

observations in no way invalidate the contentions of Vejdovský and Mencl regarding Bacterium gammari. I see no reason at present for doubting that this organism belongs to the Bacteria. My own investigations have shown merely that certain organisms, which appear to resemble B. gammari at one stage in their lives, are really not Bacteria at all, but belong to the Fungi.

Summary of Results.

I will now summarise the results which I have recorded in some detail in the foregoing pages. In this section I shall consider my own work only, without reference to the work of others. A full discussion will be found in the next section of the paper (p. 462 et seq.).

(1) All the Bacteria which I have been able to investigate with precision contain a structure (or structures) which I believe to be a nucleus. The reason for regarding these structures as nuclei is two-fold—first, from purely morphological considerations; secondly, from their staining reactions (see discussion, p. 462).

(2) The Bacteria studied belong to four different groups—namely, Cocci, Bacilli, Spirilla, and so-called "fusiform Bacteria."

(3) The Coccus forms studied possess a single, centrally placed, spherical nucleus in each cell. It divides by a simple amitosis. This type of nuclear organisation has been found in forms belonging to the genera Micrococcus and Sarcina.

(4) Cocco-bacillar forms which have been investigated show a nucleus in the form of a straight or bent rodlet, or of a more or less spiral or zig-zag filament.

(5) Bacillar forms show several different types of nuclear differentiation. The nucleus may be in the form of chromidia scattered through the cell (flexilis type, etc.); in the form of a more or less straight, spiral or zig-zag filament (spirogyra type, etc.); or in the form of irregular strands

and networks (B. saccobranchi). There is evidence to show that a nucleus in all these three forms may occur at different times in the same organism (B. saccobranchi). There is also evidence that spherical nuclei, filamentar nuclei, and chromidial nuclei may occur in the same organism at different stages in its life-history (Bacilli of modified flexilis form from Triton and Lacerta).

(6) Spirillar forms which I have studied show three different types of nucleus: the chromidial (Sp. monospora, etc.); the filamentar (Spirillum from Lacerta); and the spherical type (small Spirillum from Stylopyga), which divides by amitosis, and resembles the nucleus of Coccus forms.

(7) "Fusiform Bacteria" possess a single, usually spherical, nucleus in each cell.

(8) A number of large, parasitic, non-motile, rod-like organisms, possessing a vesicular nucleus, which appear at first sight to be Bacteria, are really Fungi allied to the yeasts.

General Discussion.

Now that I have briefly reviewed the more important literature bearing upon the cytology of the Bacteria, and have given my own observations in some detail, I am in a position to discuss my results. My main object, as I have already pointed out, has been to decide the question, whether or not the Bacteria are nucleate cells. The chief part of this discussion will therefore be directed towards answering this question.

As I have already indicated, many of the observations which have been made by others upon the cytology of the Bacteria, are based upon material which has been so imperfectly fixed and stained that it is useless to consider them. Of the researches reviewed in the "Historic" section (p. 399), therefore, only a part can be profitably considered here. Furthermore, it is impossible to enter into a minute discussion of many excellent contributions to the subject—extending,

as they do in the aggregate, over many hundreds of pages. Consequently, I crave forgiveness for the many sins of omission which must be apparent to anyone who reads the ensuing remarks.

Metachromatic Granules.—Considerable confusion exists in bacteriological literature regarding a number of granular cell-inclusions which I shall call metachromatic granules. Recent work has, however, done much to clear up this confusion, and I believe that the interpretation of these granules is now perfectly plain, and there is no cause for any further misunderstanding regarding their nature and significance. For an excellent summary of our present knowledge of these bodies, I would refer the reader to a recent paper by Guilliermond (1910).

The first to observe these granules in Bacteria appears to have been Babes. It was he, also, who subsequently named them "metachromatische Körperchen." There seems to be little doubt that the majority of colourable granules which have been described in bacterial cells really belong to this class of bodies. Different observers have given different names to the granules, and this has been largely the cause of the confusion which at present exists regarding them. It appears to me certain that the "metachromatic bodies" of Babes, the "sporogenic granules" of Ernst, the "red granules" (in part only) of Bütschli, the "chromatin granules" (in part) of Wahrlich and many others and of Meyer's earlier papers, the "granules" of Fischer, the "Volutanskugeln" of Grimme, the "volutine" granules of Meyer, the "toxigen granules" of von Behring, the "Babes-Ernst bodies" of many bacteriologists, and many other kinds of granule described by many other workers—all these are in reality the same, namely the bodies which I shall call metachromatic granules. This name aptly designates these bodies, and has been used throughout by Guilliermond[1] in his important researches into their nature; and I hope—with him—that it will find universal acceptance

[1] Guilliermond's actual name is "corpuscules métachromatiques."

and so help to clear away the confusion which now surrounds these bodies.

Metachromatic granules are found not only in many Bacteria, but also in Fungi, Algæ, Cyanophyceæ, Protozoa, and probably in many of the "higher" groups of animals and plants. Their presence in Bacteria can therefore not be used as evidence of the affinities of this group.

Regarding the chemical and staining properties of these granules, we now have a considerable mass of information—chiefly from the work of Guilliermond, Grimme and A. Meyer.[1] Their most characteristic property is that they stain red with many blue or violet stains (e.g. methylene blue, hæmatoxylin, etc.). After fixation they have a strong affinity for so-called "nuclear" stains—which has given rise to their confusion with chromatin.

Chemically considered, the metachromatic granules are probably to be regarded as composed of nucleic acid combined with an organic base (cf. Meyer, Guilliermond).

The biological significance of the metachromatic granules appears to be definitely decided. They are non-living (metaplasmic) reserve material. They are not living morphological derivatives of either nucleus or cytoplasm, but merely stored up food substance. The evidence for this appears to me overwhelming. The most important fact has been established, I believe, that they are not a constituent of the living protoplasm: they are transient, non-living elements of the cell. That they are in any way an index of the virulence of the organisms containing them, as maintained by Marx and Woithe (1900), is negatived by the work of Ascoli (1901), Krompecher (1901), Gauss (1902), Schumburg (1902), Ficker (1903), Guilliermond (1906) and others. The biological distribution of the granules throughout other organisms also speaks strongly against such a view.

It might be urged, with some justification, that the "chromidial nucleus" described in Bacteria by Schaudinn, Guilliermond and myself is really nothing more than a diffuse

[1] See also Eisenberg (1910).

system of metachromatic granules. Such a supposition has already been considered and rejected by both Schaudinn and Guilliermond. I have also had occasion already to speak against this view, and I shall now enter into it more fully.

The Bacilli of the spirogyra type which I have described, also the Micrococci, and Spirilla with a filamentar or spherical nucleus, are in the majority of cases entirely free from granular inclusions in the cytoplasm. The nuclear structures which I have described are the only constant internal structures present. It is therefore useless to argue about metachromatic granules in these forms, unless it be assumed that the nuclear filaments, etc., are metachromatic bodies—an assumption for which there is not a shred of evidence, and which is entirely opposed to the facts. It remains therefore to consider the Bacilli and Spirilla (chiefly the organisms of the flexilis type, and the Spirilla from the frog and cockroach) in which I have described a chromidial nucleus.

In the first place, I must point out that the two methods of staining—namely the Heidenhain and Romanowski methods —which I have chiefly used are not sufficient to distinguish between chromatic and metachromatic substances by means of differential staining. Both chromatic and metachromatic granules are stained black with Heidenhain and red with Romanowski. Neither method, therefore, can be used as an index of the chemical nature of the granules. In the second place, I think it highly probable that metachromatic granules do exist, side by side with the nuclear granules, in many Bacteria with chromidial nuclei (cf. also Schaudinn [1903], and Guilliermond [1908]). Bacillus flexilis itself, and also other Bacilli of the same type, contain granular inclusions which may easily be stained intra-vitam with neutral red, methylene blue and Brillantcresylblau. All these granules have a faintly reddish tinge when so treated. The same is true of Spirillum monospora. These colourable granules are few in number, however, as compared with the number visible after Heidenhain or Romanowski staining. I believe therefore that they are metachromatic

granules (reserve material) which are present in addition to the granules constituting the nuclear apparatus.

That some of the "red granules" described by Bütschli, and the "chromatin granules" of Wahrlich, A. Meyer and others are also really metachromatic granules, I think extremely probable. Yet I believe that many of these granules seen by these observers are of a nuclear nature—as in the case of my own Bacteria. Guilliermond (1908), moreover, found granules of both chromatic and metachromatic material in a number of forms which he investigated.

Now the evidence for regarding the greater part of the granules in my Bacteria as of a nuclear nature is not derived chiefly from their staining reactions—which I regard as of secondary importance—but is morphological. I shall consider this in detail in the ensuing section.

Morphological Evidence that Bacteria are Nucleate Cells.—Before proceeding any further, it is necessary to consider for a moment what is meant by the term nucleus. Various more or less unsatisfactory definitions have been given, and I do not propose to add to their number. To define any well-known thing—such as a nucleus—is merely to confine one's idea of the thing to certain arbitrarily chosen properties which it possesses, and to lay oneself open to the attacks of the verbal quibbler. It is absurd to define a nucleus in terms of certain of its chemical characteristics alone. Still more absurd is it to base a definition upon its staining reactions; for—apart from the fact that it cannot, in most cases, be definitely proved whether staining is a chemical or physical phenomenon—it is well known to every cytologist that different nuclei may display a very wide range of difference in their staining capacities. And yet I think every biologist knows what he means when he speaks about a nucleus. He means a morphological element of the living cell—a structure which could have been discovered even if chemistry were completely unknown, and staining had never been invented. The concept "nucleus" is fundamentally one of form—the idea, that is to say, belongs primarily

to the province of morphology, not of chemistry or physics. It is necessary to bear this in mind when discussing it. Hence whether a given body is a nucleus or not can only be decided by studying its morphology and then comparing it with other structures which we agree to call nuclei. Chemical properties and staining reactions may aid us materially in reaching a conclusion, but they cannot alone be used as criteria at present.[1] If they could, then a pound of nuclear substance—if it could be obtained—would be a nucleus.

One more point must be mentioned here. It has been many times asserted that Bacteria consist entirely of nucleus, or entirely of cytoplasm—because no cellular differentiation like that of other organisms has been discovered. That Bacteria are composed of cytoplasm is not frequently stated in so many words, but it is often tacitly assumed when speaking of these organisms as enucleate. But that Bacteria are nuclei has been definitely stated by many workers—especially in recent years by Ružička. Now, apart from any work which may have led to such an interpretation, I should like to point out that such statements are, *a priori*, nonsense. By "nucleus" and "cytoplasm" are meant definite *morphological* elements into which most—probably all—cells are differentiated. There is good experimental evidence that neither nucleus nor cytoplasm—specialised parts both of the living protoplasm—is capable of living independently of the other for any length of time. To call a *Bacillus* a naked *nucleus* is, therefore, a misapplication of a word in common use. An organism may have a structure similar to that of many nuclei, it may have similar chemical and staining characters,[2] but to call it a nucleus in consequence is—far

[1] In connection with the nucleus in *Bacteria* somewhat similar views have already been expressed by Schaudinn (1903). It is curious to note how many other writers are so profoundly impressed with the importance of chromatin that they frequently use "chromatin" and "nucleus" as though they were synonymous.

[2] It should also be emphasised that the "special affinity for chromatin stains," which is often attributed to Bacteria, is—as Fischer has pointed out—a myth.

from giving a satisfactory interpretation—simply to misuse words. That Bacteria are composed of a substance similar to cytoplasm may readily be granted; but to say that they consist of cytoplasm is merely to use the word "cytoplasm" in a sense which is not generally accepted. Hence, if it were proved—which it is not—that Bacteria were "cells" without a nucleus, it would be necessary to employ some other word than cytoplasm to designate their contents—for instance Van Beneden's term "plasson," or some such word. At present, however, there is no necessity to follow such a course. If one chose arbitrarily to call nucleus cytoplasm, and cytoplasm nucleus, one could easily make the astounding generalisation that cytoplasm was really not cytoplasm, but nucleus. Such, it seems to me, is the method of reasoning which is occasionally applied in considering the structure of Bacteria.

In addition to the foregoing considerations, I should like to emphasise another point. It is sometimes stated that the Bacteria show a peculiar kind of protoplasmic organisation in which nucleus and cytoplasm are not yet differentiated from one another—that Bacteria show, in fact, a primitive type of structure. Now it has never been proved —and indeed the evidence is against it—that Bacteria possess such a structure. It is obvious, therefore, that the assumption of a condition supposed to be primitive cannot be used as an argument in favour of the primitiveness of the group—as is sometimes done.

Having said so much with regard to the nucleus in general, I will pass on to an application of my reasoning to the experimental results.

I shall begin with a consideration of the Coccus forms of Bacteria. I have shown that certain Micrococci and Sarcinæ contain, in each cell, a single, centrally placed spherule. This body is a morphological feature common to every cell. When the cell divides, the spherule also divides —its division preceding that of the cell as a whole, and being characterised by the formation of a dumb-bell-shaped figure

during the process. There is therefore every reason to believe that the centrally placed body is a *living* constituent of the cell. It cannot be maintained that it is a non-living structure —for instance, a fat globule or metachromatic granule. Now on purely morphological grounds, on analogy with what is known of other cells, I think I am justified in calling this centrally placed body in cocci a *nucleus*. It corresponds as closely as could be desired with the structures which we are accustomed to call nuclei in other cells. If it is not a nucleus, then what is it? There is, I believe, only one possible answer to such a question—that it may be a structure, absent from other cells, which looks exactly like, and behaves exactly like, a nucleus, but is really not a nucleus. I think, therefore, that on morphological grounds it is completely justifiable to regard this body as a nucleus. Moreover, such a conclusion is considerably supported by the fact that the structure is stained red by Romanowski's method—the colour which is assumed by structures which are universally admitted to be nuclei.

The observations which I have made do not stand alone. They are supported by the quite independent observations of Nakanishi (1901) and Mencl (1910)[1]—both experienced workers who employed reliable cytological technique. The organisms studied by Nakanishi, Mencl and myself, though all Coccus forms of Bacteria, are all different organisms, and the cytological methods used were different in each case. Both Nakanishi and Mencl, moreover, draw the same conclusion as I do—though not altogether from the same premisses. They both believe that the structures which they discovered are nuclei.

The contention of Meyer (1908), that the nuclei described by Nakanishi are really vacuoles, is hardly worth discussing;

[1] I should like to point out—though of course I do not claim priority in the discovery of nuclei in Cocci—that my observations were in no way influenced by the work of Nakanishi or Mencl. My own observations were made before I had seen Nakanishi's work, and two years before the publication of Mencl's paper.

for the same idea occurred to Nakanishi himself, and he brought forward good experimental evidence to show that this was not the case.

As a result of my researches I regard it therefore as certain that the Coccus forms of Bacteria contain a nucleus of the form which I have described in the earlier part of this paper.

And now let us consider the other Bacteria. I have pointed out already that I have investigated a large number of cocco-bacillar organisms which present every degree of form between typical Cocci on the one hand, and typical Bacilli on the other. With change in the external shape, the nucleus shows a corresponding modification. It becomes elongated with the elongation of the cell, and hence assumes the form of a filament. In round coccus forms, the nucleus is round. In slightly elongated cocci, the nucleus is in the form of a short rodlet, which may be curved or slightly bent. In still more elongated cocco-bacillar organisms, the nucleus may have the form of a zig-zag or spiral filament. These forms merge gradually into the forms of the characteristic spirogyra type.

I have not proved that the Cocci, spirogyra Bacilli and intermediate cocco-bacillar forms, which I have found living together, are genetically connected. A proof of this is immaterial for the present purposes. It suffices to know that all these forms occur. Morphologically considered, therefore, the spiral or zig-zag filament present in Bacilli of the spirogyra type is the equivalent of the spherical body which lies in the centre of the Coccus cells. Consequently, if it is agreed that the latter is a nucleus, it follows that the spiral filament of Bacilli of the spirogyra type is also a nucleus. This is a conclusion which is supported by the behaviour of the filament during cell-division and spore-formation, which I have described in detail in B. spirogyra. My study of this form indicates beyond a doubt that the filament is a living element of the cell, and not a metaplasmic structure.

A further point in support of the morphological equivalence of the spherical nuclei of Cocci and the filamentar nuclei of certain Bacilli is furnished by the observations recorded on p. 421. I have shown that the nucleus of certain Micrococci, when the cell is elongated during the process of cell-division, may be drawn out into a zig-zag or spiral filament. We see here directly, I believe, the way in which the filamentar nucleus of some Bacilli has been derived from the spherical nucleus of Micrococcus forms.

Again, staining reactions—so far as they go—support the interpretation of the filament in Bacilli of the spirogyra type as a nucleus.

At this point Bacillus saccobranchi must be considered. I have shown that this organism possesses at one stage in its life-history a nucleus of the characteristic spirogyra type—that is to say, a spiral or zig-zag filament which is the morphological equivalent of the nucleus of Coccus forms. Now this structure undergoes a remarkable transformation during the development of the organism. It becomes converted into the form which I have called the "irregular form"—assuming an appearance of an irregularly branching filament or network. This structure in turn breaks up to form a series of granules scattered diffusely through the whole cell—the "chromidial form."[1] It follows, therefore, with absolute certainty, that if the spiral filament is a nucleus—as I have already shown is almost certainly the case—then the chromidial structures are also the morphological equivalent of a nucleus. They are developmental stages of the very same living constituent of the cell which is represented at other times by a spiral filament or irregularly branched filament or network. In Bacillus saccobranchi, therefore, there is every reason to believe that a nucleus in the form of scattered granules, or chromidia, exists at certain stages in the life-cycle.

[1] I have pointed out (p. 444) that it is possible that the changes in the nuclear structures may take place in the reverse order to that given above. It is immaterial to my argument in which direction the sequence of developmental changes takes place.

Again in this organism, staining results confirm the morphological interpretation.

Arguing now on analogy, it becomes highly probable that the scattered granules of Bacilli of the flexilis type—the chromidia, in other words—are of the same nature as the granules of Bacillus saccobranchi. They are the only morphological elements distinguishable in the cells, and that they are living structures—not reserve material—appears to me quite certain from the part which they play during spore-formation. When it is further found that, in the course of spore-formation, the granules arrange themselves in the form of a spiral or zig-zag filament[1]—like that of Bacilli of the spirogyra type—then the nuclear interpretation of the granules is not merely strengthened, but becomes almost a certainty. It appears to me that there is only one logical conclusion to be drawn from these facts—that the chromidia of Bacilli of the flexilis type represent the nucleus, being the equivalent (morphologically) of the spherical nucleus of Cocci and of the spiral filament of other Bacilli.

When we find that many smaller Bacilli show a structure which is essentially the same as that of the large Bacilli of the flexilis type, it is only natural to suppose that we see here, also, structures which are capable of a similar interpretation. The assumption is justified that the chromidia of small Bacilli constitute their nuclear apparatus.

In all these cases, moreover, staining reactions—so far as I have tried them—support the morphological interpretation.

If we now consider the structures which are present in the Bacilli of a modified flexilis form (from the newt and lizard—see p. 430) it becomes apparent that these structures also represent phases of the nuclear apparatus. The actual facts here are not so well established as in the forms which I have hitherto considered, but it is at least exceedingly probable that in these the nucleus exists, at some stages in the life-history, in the form of a few large globular masses.

[1] Discovered by Schaudinn (1902) in Bacillus bütschlii, and confirmed by me (1908) in the case of B. flexilis.

The aggregate of these masses in each cell is the morphological equivalent of the chromidia or the spiral filament.

Thus, we see here another modification of the nucleus which may exist in Bacteria.

I will now consider the spirillar forms which I have investigated. I have found that three different types of structure exist in these organisms. In one of these there is a minute spherical body present in each cell : it divides with a dumb-bell-shaped figure, its division preceding that of the cell (small Spirillum from Stylopyga). It is a living element—a morphological feature of each cell. In the second type, there is a filament of a zig-zag or spiral form, which also divides into two during cell-division (Spirillum from Lacerta muralis). Thirdly and lastly, there is a type of Spirillum whose characteristic morphological feature is a system of granules scattered through the cell (Sp. monospora, large Spirillum from Stylopyga). From a consideration of these spirillar forms alone we could, with considerable justification, reach the conclusion that these three different types of structure represent three different modifications of the nuclear apparatus—upon morphological grounds. When the analogy of these structures with the nuclei of Cocci and Bacilli is considered, however, it appears to me that only one logical deduction can be drawn, namely, that the single spherule, the spiral filament, and the chromidia of Spirilla are nuclei.

Staining, again, gives results consistent with this interpretation.

I believe my nuclear interpretation of the various structures discussed above is the only logical interpretation which can be given to the facts known to us at present. And of the accuracy of the facts which I have recorded, I have not the slightest doubt.[1]

[1] Owing to the fugitive nature of the staining methods which I have frequently employed, it is now impossible to demonstrate many of my preparations satisfactorily. I have therefore at various times demonstrated my preparations to competent observers, in order that they

It remains now to consider how far these facts coincide with those recorded by others.

First of all, I would point out that my results are in agreement with those of Schaudinn (1902, 1903) and Guilliermond (1908)—both of whom made accurate cytological investigations of different organisms. Both these observers, however, examined Bacteria which possess a nucleus of the chromidial form: it is with my chromidial forms, therefore, that their results must be compared. Both Schaudinn and Guilliermond —though on different grounds—arrived at an interpretation similar to my own.

Quite recently, Guilliermond (1909) has recorded the existence of two species of Bacillus and a Spirillum which possess nuclear filaments like those which I have described in these forms. His observations appear to have been made quite independently of mine, and may therefore be taken as confirmatory.

I find it difficult to decide how far the results of Swellengrebel (1906, 1907, 1907A, 1909, 1909A) coincide with mine. He finds in Bacilli and Spirilla remarkable filamentar structures, usually in the form of an irregular or broken spiral. On account of the micro-chemical and staining reactions of these structures, he is led to interpret them as nuclei. They are not exactly like the filamentar structures which occur in Bacilli of the spirogyra type. In many cases they resemble certain of the nuclear modifications of B. saccobranchi. It seems to me possible that in some cases also the appearances are the result of imperfect fixation[1]—the original spiral filament having been broken up in this process. Sometimes, also, the filaments may be really chromatin

could confirm my statements as to the existence of the structures which I have described—if their existence were called in question. Among those to whom I have shown one or other of my preparations may be mentioned Sir Ray Lankester, Prof. Adam Sedgwick, Prof. J. B. Farmer, and Prof. F. Vejdovský—all of whom have agreed with me as to the appearances presented.

[1] See footnote on p. 417.

granules connected by deeply stained cytoplasm—as maintained by Guilliermond (1908). As I have not myself made a study of the forms which Swellengrebel describes, and as his work has evidently been conducted with considerable care and thoroughness, I hesitate to make any more definite criticism of it at present.

The earlier observations of Bütschli (1890, 1896), Wahrlich (1890), Zettnow (1897), and others are in agreement with mine[1] if it be assumed—as appears highly probable—that they investigated only those forms of Bacteria which possess a chromidial nucleus. With Bütschli's interpretations, however, I cannot agree.

Nuclei in the form of a few small granules in each cell, described by Meyer (1897, 1899), and Preisz (1904), are probably of the same nature as chromidial nuclei, and the nuclei which occur in the Bacilli of modified flexilis type.

The facts and their interpretations, given by Nakanishi (1901) are—in many cases—closely parallel to my own. Nakanishi found filamentar nuclei in Bacilli (e.g. B. anthracis), and in Spirilla spherical and filamentar nuclei, which are very like the structures which I have myself observed in similar forms. After ably discussing his observations, Nakanishi arrived at an interpretation which agrees with mine.

How far the observations of Amato (1908) can be brought into line with my own I do not know. It is possible that the "nuclear" structures which he describes are really metachromatic granules—as suggested by Guilliermond (1910).

A point of considerable importance is to be found in the work of Schewiakoff (1893). In Achromatium, he found a number of minute chromatin granules scattered through the cytoplasm—in other words, he found a nucleus of the chromidial type. He observed that these granules undergo division—which is a further important piece of evidence

[1] So far as the actual morphology of some of the smaller Bacteria is concerned.

that they are living structures.[1] In Bacilli of the flexilis type the chromidia are too small for their division to be observed with accuracy, but I think they probably behave in much the same way as the larger chromidia of Achromatium.

The remarkable work of Mencl (1905) upon filamentous water Bacteria (Cladothrix, etc.) contains many observations which are in complete accord with mine. In the forms investigated—which are pleomorphic—he found nuclei of a spherical, filamentar, and chromidial form, with numerous intermediate forms. He was able to observe the division of these nuclei in the living cells—thus proving that they were really living structures, and not metachromatic or other non-living granules. He believes that the different nuclear forms occur, at different stages in the life-history, in the same organism. His results are therefore closely similar to mine.

The nuclear interpretation of the chromidial structures present in Bacteria—as upheld by Schaudinn, Guilliermond and myself—has been controverted by Růžička (1909) on the grounds that the whole bacterial cell is itself the equivalent of a nucleus. Apart from the a priori absurdity of this view—which I have already pointed out above—I must emphasise the fact that the observations recorded in the present paper completely condemn such an interpretation. On the other hand, I believe the chromidial view is completely vindicated. The statement made by Ambroż, who follows Růžicka, that the chromidial view has been "reduced ad absurdum" by the latter, is therefore entirely erroneous.

The observations of Mitrophanow (1893)[2] seem to me to be capable of being brought into line with my own, when allowance is made for the difference in technique. I find it not always easy to comprehend Mitrophanow's meaning; his methods of fixation and staining also seem to leave much to

[1] Hinze (1903) made similar observations in the case of Thiophysa.

[2] This paper is an abstract only of a larger work in Russian. It is therefore possible that Mitrophanow's observations and views are more clearly given in the original—which is unfortunately inaccessible to me.

be desired. Nevertheless, he appears to have found organisms possessing nuclei in the form of chromidia, spiral filaments and spherical masses of chromatin. He also distinguishes between nuclei and "granulations," and points out the structural variability which the nucleus displays. On the whole, his observations—so far as I understand them—appear to be in agreement with mine.

Kunstler's (1887) observations upon the structure of Spirilla agree closely with what I have myself described in Spirilla with a chromidial type of nucleus. Also the chromatin structures described in the cholera Vibrio by Podwyssozki (1893) bear a strong resemblance in many cases to the nuclei which I have shown to occur in the small Spirillum from the gut of Stylopyga orientalis.

I believe the "chromatin" granules described in sulphur Bacteria by Hinze (1901, 1903) and Dangeard (1909) are—like Bütschli's findings in similar forms—to be interpreted as nuclei in a chromidial condition. The same interpretation will apply to the granules of B. oxalaticus, described by Migula (1894); and also to the iron- and phosphorus-containing granules found in Beggiatoa by Macallum (1899).[1]

Rowland's (1899) results can easily be explained if it be supposed that the organisms which he studied possessed nuclei in the form of chromidia in addition to metachromatic bodies.

I think I may fairly claim, from what I have already pointed out in the preceding pages, that not only do my own observations furnish most conclusive evidence with regard to the nucleus in Bacteria, but that in almost every case in which careful investigation has been made by others, the results are not inconsistent with mine. In many cases they are, indeed, completely confirmatory. When good technique has been employed, and careful observations have been made,

[1] Certain points in connection with fixation are, moreover, not quite clear to me in the work of this author. It may also be pointed out that Macallum failed to find a nucleus in the yeasts—in which a typical vesicular nucleus certainly occurs.

I do not believe a single fact of any importance has been found which speaks against my results. In matters of interpretation, of course there is considerable difference of opinion already existing; but I am convinced that no interpretation, other than that which I have given, can be found which will fit all the facts known to us at present. How far such a conviction is justified further work alone can show.

So far I have considered only the Bacteria themselves, and I believe the evidence which I have given from this group alone is sufficient to establish the fact that Bacteria are nucleate cells. Considerable additional evidence may, however, be adduced from analogy with two other groups of organisms—the Protozoa and the Cyanophyceæ. In the Protozoa, a chromidial form of nucleus occurs in many different organisms, as a transient stage in the life-cycle. It may also occur as the normal vegetative condition. It is unnecessary to enter into this subject in detail here. The reader will find a condensed account of chromidia in a paper which I have previously published (see Dobell, 1909B). A nucleus in the form of irregular strands, networks, granules, etc., scattered through the cytoplasm, also occurs in Protozoa—especially in the Infusoria (cf. Dobell, 1909A).

In the Cyanophyceæ, analogous nuclear conditions probably obtain. It is impossible in the present paper to enter into a discussion of the vexed question of the nucleus in this group, but I should like to call attention to two recent contributions to the subject which have been made as a result of careful cytological work. I refer to the work of Gardner (1906) and Guilliermond (1907A) Gardner describes and figures nuclei in the form of networks, granules, and irregularly branched filaments. Guilliermond describes similar structures, and also nuclei in Nostoc which resemble those of Micrococci, and nuclear filaments, like those of Bacillus spirogyra, in Rivularia. If analogies were wanting for the structures which I believe to be nuclei in Bacteria, they could be found therefore without any great difficulty in the nuclei of other organisms.

Do Bacillar Forms with a Vesicular Nucleus exist?—I have already had occasion to note that Bacilli with a typical vesicular nucleus have never come under my observation. All the organisms which I found to be constituted in this manner have proved to be Fungi. Others, however, have described very definite instances in which vesicular nuclei occur, and the matter is of such moment that a brief discussion is here necessary.

In the accounts of the older observers, the observations are so incomplete, and the technique employed was so imperfect, that a discussion seems useless. This is not the case with some more recent work, however. I refer to the publications of the Bohemian investigators, Vejdovský, Mencl, and Raýman and Kruis.

There seems no doubt at all, from the very careful work of Vejdovský (1900, 1904) and Mencl (1907), that the organism which the former has named Bacterium gammari really possesses a vesicular nucleus, which divides mitotically.[1] The only point which requires to be settled is whether the organism really belongs to the Bacteria or not. Considerable discussion has already taken place regarding this. Some observers (e. g. Guilliermond, 1907, 1908, 1910) are inclined to regard it as a yeast-like fungus—not a Bacterium at all. The resemblance between certain yeast forms and this organism is certainly very striking (compare, for example, the figures of Mencl [1907]—figs. 4, 7, 10, etc. [pl. x]—with Wager's [1898] figures—figs. 45, 46, 47 [pl. xxx]—of Saccharomyces pastorianus). After my own experiences with Bacterium-like yeasts (see p. 455), I hesitate to express an opinion with regard to B. gammari. It is most important that further observations should be made upon this most interesting organism; and it is to be hoped that

[1] My friend Prof. Vejdovský has very kindly given me a preparation of this organism, so that I have been able to examine it myself. To my mind there can be no doubt as to the accuracy of the accounts which have been given of it.

before long someone to whom fresh material is accessible will reinvestigate the matter thoroughly.[1]

Regarding Vejdovský's filamentar forms from Bryodrilus, my opinion is that they are really Fungi, similar to those which I have myself described. Guilliermond (1907, 1908) expresses a similar opinion—"nous sommes à peu près certains, après l'examen attentif de ses (i.e. Vejdovský's) préparations, qu'elle correspond à une moisissure. Nous n'avons trouvé en tous cas, dans cette espèce aucun des charactères des Bactéries" (1908, p. 37).

I think there can be no doubt that the Bacterium-like organisms, which I have already described (p. 455), are really Fungi, allied to the Saccharomycetes. The evidence for this is chiefly derived from two features of their life history—(1) the assumption of a characteristic yeast form, which reproduces by budding, (2) the formation of mycelium-like outgrowths.[2] Similar outgrowths have been observed in yeasts by other workers (cf. Janssens and Mertens, 1903). To this same group of organisms belong—I believe—two other forms which have recently been described, namely, Kermincola, a parasite of the body cavity of Coccid insects (Šulc, 1906), and Bacillopsis stylopygæ, from the cockroach (Petschenko, 1908). Both these forms appear to me to be indubitably Fungi, and not Bacteria (cf. also Vejdovský, 1906). The fact that my organisms, Kermincola and

[1] It is to be gathered from the discussion which has taken place regarding B. gammari (Guilliermond, 1907, 1910; Mencl, 1909) that Schaudinn—who saw Vejdovský's preparations at the Zoological Congress in Berne—at first expressed the opinion that the organism was a yeast. Later, however, he accepted Vejdovský's interpretation of it as a Bacterium—an opinion shared also by Schewiakoff.

[2] The formation of outgrowths is of course occasionally observable in true Bacteria (Bacilli, Bacteria, Spirilla). It is usually observed only in involution forms. Meyer (1901) interprets the outgrowths as a reminiscence of mycelium formation in the ancestors of Bacteria—believing them to be of fungal origin. For my own part, I do not believe that the Bacteria have anything whatever to do with the Fungi, and do not regard this as a correct interpretation of the phenomenon.

Bacillopsis, are all Fungi, indicates of course nothing regarding the existence or non-existence of true Bacteria with a typical vesicular nucleus.

The vesicular nuclei described in Bacteria by Mencl (1904, 1905, 1907) and Raýman and Kruis (1904) are, according to Guilliermond (1907, 1908, 1910), capable of a very different interpretation. According to him, the "nuclei" are really nothing more than various stages in the formation of transverse septa in dividing cells. This interpretation is vigorously attacked by Mencl (1909), who maintains that vesicular nuclei are actually present, and can be readily distinguished from the transverse septa. Mencl's figures certainly seem clear enough—as do the photographs of Raýman and Kruis. And I find it difficult to believe that so accurate and experienced an observer as Mencl could make such a mistake. Swellengrebel's (1907) results on B. binucleatum are also favourable to his interpretations. Yet a certain amount of uncertainty exists at present regarding these forms.

Finally I must say that it seems to me probable that Bacteria do exist which possess—at any rate during part of their life-cycle—nuclei of the vesicular form characteristic of the cells of "higher" animals and plants. It is certainly not legitimate to argue that because Bacteria have not been previously found which contain a vesicular nucleus, therefore that any form in which a vesicular nucleus can be demonstrated —e.g. Bact. gammari—does not belong to the Bacteria, but to the Fungi or some other group. This is simply begging the question. There is absolutely no reason, either from my own observations or from those of other workers, why typical vesicular nuclei should not occur in some Bacteria. The evidence, in fact, is in favour of the view that such nuclei do exist in certain Bacteria at certain stages in their lives.

Variability of the Nucleus at different Periods in the Life-cycle.—It will already be apparent to anyone who has read the preceding part of this paper, that the nucleus of any given bacterium is not necessarily constant in its form at

all stages in the life history. This point seems to me worth special attention.

In the case of Bacillus saccobranchi, I have pointed out that the nucleus may be in the form of a spiral filament, or in the form of chromidia, or in forms intermediate between these and characterised by having an appearance of irregular strands, granules or networks of chromatin. There can be no doubt that, in this Bacillus at least, the nucleus has a variable structure. There is, however, no evidence to show what relations these various nuclear modifications bear to the life-cycle as a whole. All that can be said at present is that these different nuclear forms exist.

When we turn to the Bacilli of the flexilis type, however, we have exact knowledge of the relations between the nuclear modifications and the phases in the life-cycle. From Schaudinn's (1902) study of B. bütschlii and my own researches on B. flexilis and allied forms it can be definitely stated that the chromidial stage represents the normal vegetative condition of the nucleus, existing throughout the greater part of life. A nucleus in the form of a spiral filament occurs as a transient stage connected with, and immediately preceding, spore-formation. In the spore itself a third nuclear modification is seen. The chromatin is in the form of a densely aggregated mass, which constitutes the chief part of the living substance of the spore. From this aggregated mass the chromidial condition is again assumed in the process of germination from the spore.

In the Bacilli which I have termed those of a "modified flexilis form," these three nuclear conditions are encountered in a modified form, but their relation to the phases of the life-cycle has not been determined.

In Spirillum monospora (Dobell, 1908), Bacillus sporonema (Schaudinn, 1903) and many other Bacilli (Guilliermond, 1908) only two modifications of the nucleus have been established. During the vegetative condition the nucleus is in the form of chromidia. It then assumes the form of an aggregated mass, which enters into the formation

of the spore. These two different nuclear conditions therefore coincide very definitely with two different phases of the life-cycle.

In Bacillus spirogyra and allied organisms—as I have shown (1909)—two nuclear conditions are also found.[1] In the ordinary vegetative part of the life-history the nucleus is in the form of a filament. A part of this gives rise to a large, aggregated spherical mass of chromatin which enters into the spore. Here, again, the nuclear changes are correlated with definite stages in the life-history. I have not studied the young Bacilli which emerge from the spores in any organism of the spirogyra type. I cannot therefore state with certainty that the observed nuclear changes are the only ones which exist. On analogy with B. saccobranchi, it is quite possible that a chromidial condition of the nucleus occurs in Bacteria of this sort.

I have shown that three different nuclear conditions exist in three different species of Spirilla which I have studied. If one can argue on analogy in this case, it appears not improbable that these nuclear conditions are temporary, and that other phases in the nuclear structure exist in these organisms also. It is quite possible, for example, that the nuclear filament in the Spirillum from the intestine of Lacerta muralis may at other stages in the life-cycle—as in Bacillus saccobranchi—become modified into the chromidial form of nucleus which exists in such an organism as Sp. monospora.

My own belief is that the nucleus in Bacteria may display not one, but many forms during the whole life-cycle. Many of the nuclear structures which have been shown to exist in these organisms should, I think, be regarded as temporary stages rather than as permanent conditions. The different results which have been reached

[1] It may be emphasised also that the spiral filament itself in Bacteria of this type shows a wide latitude of variation in form. Whether these variations are correlated with special stages in the life-cycle is as yet unknown.

by different workers when working, apparently, upon the same species, may to some extent find an explanation in this circumstance.

I would call attention to the fact that Mencl[1]—whose studies have been carried on with quite different Bacteria from those which I have investigated—has arrived at a similar conclusion. Many times Mencl has emphasised this point—a point which is, I believe, of fundamental importance for reaching a correct interpretation of the Bacteria. I am rejoiced that in this we are both agreed.

Pleomorphism.—Though I have no conclusive evidence to add to what has already been contributed to the hypothesis of the pleomorphism of Bacteria, nevertheless, I must point out that many of the facts recorded in the earlier part of this paper are consistent with such a view.

Whilst investigating the Micrococcus, Cocco-bacillus and Bacillus forms which I found in the gut of the lizard, I was often impressed by the apparent genetic relations existing between them. The same was the case with many of the different bacillar forms which I found in the blood of Saccobranchus. I have already pointed this out in previous pages, and although a direct proof of such genetic continuity is wanting, my observations are completely in accord with such an interpretation. This appears to me, in fact, the most probable hypothesis at present: otherwise it would be necessary to assume the existence of an almost inconceivably large number of species to account for the number of intermediate forms which occur.

For my own part, I believe—although this is a view which is not held by the majority of "bacteriologists"—that the greater number of Bacteria are pleomorphic. That pleomorphism does exist in many Bacteria, I think there can be no longer any doubt. Since the early work of Ray Lankester, Cienkowski, Zopf, Metchnikoff and others, an immense mass of evidence has been brought forward in favour of such a view. It is outside the limits of the present paper to enter

[1] See especially his studies on water Bacteria (Mencl, 1905).

into a discussion of this matter, but I should like to call attention to the exhaustive—but almost completely ignored —work of Billet (1890), and the remarkable researches of Mencl (1905) in this connection. Here will be found an immense collection of facts bearing upon the matter.

It appears to me probable that—just as in the case of their nuclei—the majority of Bacteria may possess a wide range of variation in their outward form at different stages in their life-histories. The matter can be decided, however, by further research only: but it offers a vast field for future investigation—investigation which is not only of a most fascinating nature, but of which the results also will be of the greatest biological interest.

Do Enucleate Bacteria Exist?—I wish to say a few words here about the belief which is often held, that the Bacteria are a group of organisms which possess no structure homologous with the nucleus present in the cells of other protists, animals or plants.

From a survey of the work which has been done upon the cytology of the Bacteria, I think it may be stated with absolute certainty that not a single bacterial species has been proved to be devoid of a nucleus. I do not say that a nucleus has been proved to be present in every bacterial species: but I do maintain that a nucleus has been demonstrated in a large number of species of Bacteria. The probability is, therefore, that all Bacteria are nucleate cells. That enucleate Bacteria may exist, is, of course, a possibility which cannot be denied; but at present there is absolutely not a vestige of evidence in favour of such a view.

I should like also to draw attention to a sort of statement about Bacteria which may be very frequently encountered in biological writings. The following quotation will serve as an instance of the sort of thing I mean: "It may be pointed out that it is in these low forms of life that we must look for a key to the secret of the origin of the cell nucleus, as well as for data to determine the morphological character of the

primal life organism" (Macallum, 1899, p. 439). This is one case in which this idea is definitely stated, but dozens of other passages in the works of other writers can easily be found in which a similar view is either formulated or tacitly assumed.

In statements of this sort two assumptions are made: first, that Bacteria are more simply organised than other living beings; secondly, that the more simply organised beings are phylogenetically the more primitive. There is no real justification for either of these assumptions. By calling Bacteria "low forms of life," it is easy enough to arrive at the conclusion that they occupy a position near the bottom of the phylogenetic tree. But this is nothing more than a petitio principii—a using of the conclusion at which it is desired to arrive as evidence for that conclusion. It is, of course, open to anybody to make the assumption that the Bacteria are like the most primitive forms of life; but the fact should not be lost sight of that this is at present an assumption, and nothing more.

"Fusiform Bacteria."—All the so-called "fusiform Bacteria" which I have examined possess a distinct nucleus, usually in the form of a spherical mass of chromatin—one in each cell. This nucleus divides previous to the division of the cytoplasm.

Nuclei, which divide by amitosis, were originally described in the fusiform organism ("Bacillus fusiformis") which occurs in the human mouth, by Mühlens and Hartmann (1906). This—so far as I am aware—was the first record of nuclei in these organisms. A detailed description of the nucleus was not given, and no figures were published.

Quite recently, Hoelling (1910) has given a detailed account of a fusiform organism—which he names Fusiformis termitidis[1]—which occurs in the gut of termites (locality and species not stated). He also describes and

[1] Presumably a mistake for termitis. Hoelling proposes for all the fusiform organisms the generic name Fusiformis in place of the obviously inapplicable name Bacillus.

figures the fusiform organism from the human mouth, a form from fresh water, and a form from the cæcum of a mouse. In all these, he finds nuclei which are essentially the same as those which I have found in the various forms described in the preceding pages.

Hoelling describes the formation of long, multinuclear filaments by these organisms. He regards this as a degeneration phenomenon. The occurrence of these filamentar (unsegmented) forms lends, I think, some support to the view, which I have already expressed (p. 452), that the "fusiform Bacteria" are really Fungi.[1] At present there is no conclusive proof that this is so; but it should be noted also that there is no proof that these protists are Bacteria.

Whatever be the systematic position of the "fusiform Bacteria," I think there can be no longer any doubt that they possess a characteristic nucleus, in the form usually of a minute sphere or granule—one in each cell—which divides by a simple process of amitosis.

Affinities of the Bacteria.—This is not the place to discuss the affinities of the Bacteria in detail. Yet I believe we have now arrived at the beginnings of a correct interpretation of the structure and life-history of this group, so that a discussion of their affinities would be more profitable now than it would have been a few years ago.

Three chief views regarding the affinities of the Bacteria have been advanced: namely, that they are allied to the Fungi, to the Cyanophyceæ, or to the flagellate Protozoa. I have previously expressed the opinion that the Bacteria do not show affinities with the Fungi. The cytological studies recorded in this paper confirm this view completely. I believe there is not a particle of evidence to support the hypothesis that the Bacteria and Fungi are connected. The

[1] I would call attention to the resemblance which these organisms bear to a fungus described by Šulc (1910) from the body-cavity of Chermes strobilobius. This fungus—probably a yeast—which Šulc calls Schizosaccharomyces chermetis strobilobii, has a "caraway-seed shape," and the figures of it (fig. xv) certainly show a strong similarity to many "fusiform Bacteria" which I have observed.

name "Schizomycetes"—or "Spaltpilze"—is a complete misnomer. Similarly, with regard to the Protozoa, I see no real evidence at all which indicates that affinities exist between this group and the Bacteria. There is no real similarity between them.

There is, perhaps, rather more evidence of the affinities of the Bacteria with the Cyanophyceæ. Nuclear resemblances between the two groups certainly do exist, but on the other hand there are many important differences. The evidence is certainly very far from conclusive.[1]

I believe that at present there is no clear evidence of the affinity of the Bacteria with any other group of organisms. For the present they must be regarded as a group of Protista which stands quite apart.

I believe, further, that amongst the Bacteria a number of forms are included which do not really belong—that the group Bacteria, as at present constituted, comprises a very heterogeneous assemblage of forms.

Similar views to these have already been expressed by Mencl (1907) and Guilliermond (1907), when considering the facts which were then known. I have myself also expressed the same views on a previous occasion, and I believe that they are now completely justified.

Conclusions.

I think, from the facts which have been given and analysed in the foregoing pages, the following chief conclusions are justified:

All Bacteria which have been adequately investigated are—like all other Protista—nucleate cells.

[1] I should like to point out here that the cytology of the Cyanophyceæ and sulphur Bacteria does not furnish us with anything more than analogical evidence regarding the structure of the smaller Bacteria (i.e. Bacilli, Spirilla, etc.). I believe many sulphur Bacteria are probably only distantly related to the majority of the smaller forms, and there is no clear evidence that the Cyanophyceæ have anything to do with them.

The form of the nucleus is variable, not only in different Bacteria, but also at different periods in the life-cycle of the same species.

The nucleus may be in the form of a discrete system of granules (chromidia); in the form of a filament of variable configuration; in the form of one or more relatively large aggregated masses of nuclear substance; in the form of a system of irregularly branched or bent short strands, rods, or networks; and probably also in the vesicular form characteristic of the nuclei of many animals, plants, and protists.

There is no evidence that enucleate Bacteria exist.

Finally, in addition to these purely morphological conclusions concerning the nucleus, I think another conclusion is rendered highly probable:

The Bacteria are in no way a group of simple organisms, but rather a group displaying a high degree of morphological differentiation coupled in many cases with a life-cycle of considerable complexity.

Appendix.

On the Alleged Autogamy of Bacteria.—In two earlier papers I have discussed the so-called "autogamy" of the disporic Bacteria in some detail. The actual facts regarding this process were recorded by Schaudinn (1902, 1903), and myself (1908). In a second paper (1909) I brought forward strong evidence to show that the so-called "autogamy" of Bacteria is not a sexual process at all, but has a much simpler explanation. It seems necessary, however, to refer to this matter once more, owing to the recent appearance of a very misleading article by Dr. Růžička.[1]

After mentioning Schaudinn's observations, the author

[1] V. Růžička, "Ueber die experimentelle Autogamie der Bakterien," 'Arch. Entw.-Mech.,' Bd. xxx. Festschrift f. W. Roux, Teil. 1, p. 443, 1910.

proceeds (p. 443)—"Eine Bestätigung dieser Befunde ist bis jetzt nur von Dobell[1] eingelaufen, und zwar insofern, als er bei Bac. flexilis zum Teil ähnliche Bilder vorgefunden hat. Er bestreitet indes die Deutungen Schaudinns, weil er die von diesem Forscher geschilderten und seine Deutung eigentlich bedingenden Plasmaströmungen nicht beobachten konnte." And further (p. 445)—"Vielleicht ist der negative Befund Dobells damit zu erklären, dass er ohne vitale Färbung untersucht hat."

Now if Dr. Růžička had taken the trouble to read my first paper, he would have found that my results were essentially the same as Schaudinn's; that I accepted then Schaudinn's interpretation that the phenomenon was probably a sexual one; and that I did employ intra-vitam staining methods, and was unable to convince myself that streaming of the granules occurred in the living organisms on account of their motility.[2] It is in my second paper (1909)—which Dr. Růžička completely ignores—that I have given what is, I believe, a definite proof that no sexual process occurs during spore-formation in the disporic Bacteria. There is very strong evidence that the "sexual" phenomena are due simply to a suppressed cell-division. I should like to point out that Dr. Růžička's own observations, recorded in this paper, support my view. The "sexual act" which he invoked by growing his Bacteria upon abnormal and innutritious media may be quite simply explained by the fact—which he himself records—that the organisms divided imperfectly and then proceeded to form spores without developing typical colonies. Dr. Růžička's incomplete observations and figures of the formation of disporic individuals add nothing to the facts observed and recorded by Schaudinn and myself. Disporic, or coupled monosporic, individuals have already been observed in many different Bacteria by many workers.

[1] Here follows a reference to my 1908 paper.

[2] But I have never used this as an argument against the sexual interpretation of the phenomenon. That some of the granules do pass to the ends of the cells I have, I think, helped to prove.

As Dr. Ružička has added no new facts regarding the method of spore-formation in these alleged autogamic forms, it is only his interpretation of the phenomena that I can dispute. But as I have already given my arguments against the view which he adopts, I can suffice with referring him to my second (1909) paper.

One or two other points appear worthy of mention. Dr. Ružička says (p. 443)—"Die Bakterien, bei welchen man bislang geschlechtliche Vorgänge festgestellt hat, waren als zufällige Gäste oder Parasiten andrer Organismen vorgefunden worden, ohne weiter und reingezüchtet worden zu sein. Das hätte Skeptikern als Punktum fixum dienen können, um ihre Zweifel an der Reihenfolge der Phasen des besprochenen Vorganges und an seiner Zugehörigkeit zu den sexuellen Erscheinungen weiter zu spinnen." Now it may be noted, in the first place, that B. sporonema is a free-living form; and secondly, that phenomena continuously observed in organisms in their natural environment are of more, or at least equal, importance to those observed under abnormal conditions, in which many of the factors are unknown.

Dr. Ružička concludes his paper by stating (p. 458) that the facts of the alleged "autogamic" process are in accord with his interpretation of Bacteria as nuclei. It seems scarcely necessary to point out that such an opinion could be arrived at only by a complete confusion of ideas coupled with a misuse of words.

It seems to me unnecessary to discuss the speculative part of Dr. Ružička's paper, since it is based—I believe—upon his misinterpretation of the facts. Until it can be proven that sexual phenomena occur, it is useless to construct further speculations upon the mere assumption. And at present I believe all the evidence speaks very definitely against the view that a sexual process occurs at any stage in the life-history of Bacteria.

Imperial College of Science and Technology,
London. October 5th, 1910.

Literature.

Amato, A. (1908).—"Ueber die feine Struktur der Bakterien," 'CB. Bakt.,' Abt. i, Bd. xlviii, p. 385.

Ambrož, A. (1909).—"Entwicklungscyclus des Bacillus nitri sp. n. als Beitrag zur Cytologie der Bakterien," 'CB. Bakt.,' Abt. i, Bd. li, p. 193.

Ascoli, G. (1901).—"Zur Morphologie der Bakterien und ihre Beziehung zur Virulenz," 'Deutsch. med. Wochenschr.,' No. xx, p. 313.

——— (1901A).—"Bemerkungen, über den Bau der Bakterien—von Dr. K. Nakanishi," 'CB. Bakt.,' Bd. xxx, p. 910.

Babes, V. (1889).—"Ueber isolirt färbbare Antheile von Bakterien," 'Zeitschr. Hyg.,' Bd. v, p. 173.

——— (1895).—"Beobachtungen über die metachromatischen Körperchen, Sporenbildung, Verzweigung, Kolben- und Kapselbildung pathogener Bakterien," 'Zeitschr. Hyg.,' Bd. xx, p. 412.

De Bary, A. (1884).—'Vergleichende Morphologie und Biologie der Pilze, Mycetozoen und Bacterien,' Leipzig (Engelmann).

Billet, A. (1890).—"Contribution à l'étude de la morphologie et du developpement des Bactériacées," 'Bull. sci. France et Belgique,' t. xxi, p. 1.

Bütschli, O. (1890).—'Ueber den Bau der Bacterien und verwandter Organismen,' Leipzig (Winter).

——— (1892).—'Untersuchungen über mikroskopische Schäume und das Protoplasma,' Leipzig.

——— (1896).—'Weitere Ausführungen über den Bau der Cyanophyceen und Bacterien,' Leipzig (Engelmann).

——— (1902).—"Bemerkungen über Cyanophyceen und Bacteriaceen," 'Arch. Protistenk.,' Bd. i, p. 41.

Dangeard, P. A. (1909).—"Note sur la structure d'une Bactériacée, le Chromatium okenii," 'Bull. Soc. bot. France,' t. lvi, p. 291.

Dietrich, A. (1904).—"Ueberblick über unsere Kenntnisse von der Morphologie und Biologie der Bakterien (Sammelreferate)," 'Zeitschr. allg. Physiol.,' Bd. iii, p. 23.

Dobell, C. C. (1908).—"Notes on some Parasitic Protists," 'Quart. Journ. Micr. Sci.,' vol. 52, p. 121.

——— (1909).—"On the so-called 'Sexual' Method of Spore-formation in the Disporic Bacteria," 'Quart. Journ. Micr. Sci.,' vol. 53, p. 579.

——— (1909A).—"Some Observations on the Infusoria parasitic in Cephalopoda," 'Quart. Journ. Micr. Sci.,' vol. 53, p. 183.

Dobell, C. C. (1909B).—"Chromidia and the Binuclearity Hypotheses: a Review and a Criticism." 'Quart. Journ. Micr. Sci.,' vol. 53, p. 279.

Eisenberg, P. (1910).—"Ueber Nilblaufärbung zum Nachweis der metachromatischen Bakteriengranula." 'CB. Bakt.,' Abt. i, Bd. liii, p. 551.

Ernst, P. (1888).—"Ueber den Bacillus xerosis und seine Sporenbildung." 'Zeitschr. Hyg.,' Bd. iv, p. 25.

——— (1889).—"Ueber Kern- und Sporenbildung bei Bakterien." 'Zeitschr. Hyg.,' Bd. v, p. 428.

——— (1902).—"Ueber den Bau der Bakterien (Ergebnisse vitaler Färbung)." 'CB. Bakt.,' Abt. ii, Bd. viii, p. 1.

Errera, L. (1902).—"Sur une Bactérie de grandes dimensions: Spirillum colossus." 'Rec. Inst. bot. Univ. Bruxelles,' t. v, p. 347.

Fedorowitsch, A. (1902).—"Ueber die Körnigkeit der Bakterien." 'CB. Bakt.,' Abt. ii, Bd. viii, p. 481.

Feinberg (1900).—"Ueber den Bau der Bakterien." 'CB. Bakt.,' Abt. i, Bd. xxvii, p. 417.

Ficker, M. (1903).—"Zur Frage der Körnchen und Kerne der Bakterien." 'Arch. Hyg.,' Bd. xlvi, p. 171.

Fischer, A. (1894).—"Untersuchungen über Bakterien." 'Jahrb. wiss. Bot.,' Bd. xxvii, p. 1.

—— (1897).—'Untersuchungen über den Bau der Cyanophyceen und Bakterien,' Jena (Gustav Fischer).

—— (1899).—'Fixirung, Färbung und Bau des Protoplasmas,' Jena (Gustav Fischer).

—— (1903).—'Vorlesungen über Bakterien,' 2 Aufl., Jena.

Frenzel, J. (1891).—"Der Zellkern und die Bakterienspore," 'Biol. Centralbl.,' Bd. xi, p. 757.

——— (1892).—"Ueber den Bau und die Sporenbildung grüner Kaulquappenbacillen." 'Zeitschr. Hyg.,' Bd. xi, p. 207.

Gardner, N. L. (1906).—"Cytological Studies in Cyanophyceæ." 'Univ. California Pub. (Botany),' vol. ii, No. 12, p. 237.

Gauss, C. J. (1902).—"Babes-Ernstsche Körperchen und Virulenz bei Bakterien," 'CB. Bakt.,' Abt. i, Bd. xxxi, p. 92.

Georgevitch, P. (1910).—"Bacillus thermophilus Jivoini nov. spec. und Bacillus thermophilus Losanitchi, nov. spec." 'CB. Bakt.,' Abt. ii, Bd. xxvii, p. 150.

Giemsa, G. (1909, 1910).—"Färbung von Feuchtpräparaten und Schnitten mit der Azureosinmethode." usw. 'Deutsch. med. Wochenschr.,'

No. 40, 1909; ibid., No. 12, 1910; 'CB. Bakt.,' Abt. i, Bd. liv, 1910, p. 489.

Grimme, A. (1902).—"Die wichtigsten Methoden der Bakterienfärbung und ihre Wirkung auf die Membran, den Protoplasten und die Einschlüsse der Bakterienzelle," 'CB. Bakt.,' Abt. i, Bd. xxxii, p. 1.

Guilliermond, A. (1906).—"Les corpuscules métachromatiques ou grains de volutine," 'Bull. Inst. Pasteur,' t. iv, p. 145.

——— (1907).—"La cytologie des Bactéries," 'Bull. Inst. Pasteur,' t. v, p. 273.

——— (1907A).—"Contribution à l'étude cytologique des Cyanophycées," 'Rev. gén. Bot.,' t. xviii, p. 392.

——— (1908).—"Contribution à l'étude cytologique des Bacilles endosporés," 'Arch. Protistenk.,' Bd. xii, p. 9.

——— (1909).—"Observations sur la cytologie d'un Bacille," 'CR. Soc. Biol.,' t. lxvii, p. 102.

——— (1910).—"A propos de la structure des Bacilles endosporés. Reponse à M. E. Mencl," 'Arch. Protistenk.,' Bd. xix, p. 6.

——— (1910A).—"A propos des corpuscules métachromatiques ou grains de volutine," 'Arch. Protistenk.,' Bd. xix, p. 289.

Hinze, G. (1901).—"Ueber den Bau der Zellen von Beggiatoa mirabilis Cohn," 'Ber. deutsch. bot. Ges.,' Bd. xix, p. 369.

——— (1903).—"Thiophysa volutans, ein neues Schwefelbakterium," 'Ber. deutsch. bot. Ges.,' Bd. xxi, p. 309.

Hoelling, A. (1907).—"Spirillum giganteum und Spirochæte balbianii," 'CB. Bakt.,' Abt. i, Bd. xliv, p. 665.

——— (1910).—"Die Kernverhältnisse von Fusiformis termitidis. 'Arch. Protistenk.,' Bd. xix, p. 239.

Hueppe, F. (1886).—'Die Formen der Bakterien und ihre Beziehungen zu Gattungen und Arten,' Wiesbaden.

Ilkewicz, W. (1894).—"Ueber die Kerne der Milzbrandsporen," 'CB. Bakt.,' Bd. xv, p. 261.

Janssens, F. A., and Mertens, A. (1903).—"Étude microchimique et cytologique d'une Torula rose," 'La Cellule,' t. xx, p. 353.

Krompecher, E. (1901).—"Untersuchungen über das Vorkommen metachromatischer Körnchen bei sporentragenden Bakterien und Beiträge zur Kenntnis der Babes-Ernst'schen Körperchen," 'CB. Bakt.,' Abt. i, Bd. xxx, p. 385.

Kunstler, J. (1887).—"Contribution à la technique des Bactériacées," 'CR. Acad. Sci. Paris,' t. cv, p. 684.

Kunstler, J. (1900).—"Remarques sur certains points de l'histoire de la vie des organismes inférieurs," 'CR. Acad. Sci. Paris,' t. cxxx, p. 1416.

——— and Busquet, P. (1897).—"Sur la valeur nucléaire du corps central des Bactériacées," 'CR. Acad. Sci. Paris,' t. cxxv, p. 1112.

——— ——— (1898).—'Observations sur la structure des Bactériacées et des organismes voisins,' Bordeaux (Imp. du Midi).

——— and Gineste, C. (1906).—"Spirillum periplaneticum, nov. spec.," 'CR. Soc. Biol.,' t. lviii (2), p. 135.

——— ——— (1906A).—"Structure fibrillaire chez les Bactériacées," 'CR. Acad. Sci. Paris,' t. cxliii, p. 84.

Loewit, M. (1896).—"Zur Morphologie der Bakterien," 'CB. Bakt.,' Abt. i, Bd. xix, p. 673.

Macallum, A. B. (1899).—"On the Cytology of Non-nucleated Organisms," 'Trans. Canadian Inst., Toronto,' vol. vi, p. 439.

Mann, G. (1902).—'Physiological Histology, Methods and Theory,' Oxford (Clarendon Press).

Marpmann, G. (1900).—"Ueber kernlose Bakterien," 'CB. Bakt.,' Abt. ii, Bd. vi, p. 673.

Marx, H. (1902).—"Einige Bemerkungen zu Krompecher's Arbeit über metachromatische Körnchen und Babes-Ernstsche Körperchen," 'CB. Bakt.,' Abt. i, Bd. xxxi, p. 107.

——— and Woithe, F. (1900).—"Morphologische Untersuchungen zur Biologie der Bakterien," 'CB. Bakt.,' Abt. i, Bd. xxviii, p. 1.

Menel, E. (1904).—"Einige Beobachtungen über die Struktur und Sporenbildung bei symbiotischen Bakterien," 'CB. Bakt.,' Abt. ii, Bd. xii, p. 559.

——— (1905).—"Cytologisches über die Bakterien der Prager Wasserleitung," 'CB. Bakt.,' Abt. ii, Bd. xv, p. 544.

——— (1907).—"Nachträge zu den Strukturverhältnissen von Bacterium gammari Vejd.," 'Arch. Protistenk.,' Bd. viii, p. 259.

——— (1907A).—"Eine Bemerkung zur Organisation der Periplaneta-Symbionten," 'Arch. Protistenk.,' Bd. x, p. 188.

——— (1909).—"Die Bakterienkerne und die 'cloisons transversales' Guilliermond's," 'Arch. Protistenk.,' Bd. xvi, p. 62.

——— (1910).—"Ueber den Kern und seine Teilung bei Sarcinen und Micrococcus ochraceus (butyricus)," 'Arch. Protistenk.,' Bd. xix, p. 127.

Meyer, A. (1897).—"Studien über die Morphologie und Entwickelungsgeschichte der Bakterien, ausgeführt an Astasia asterospora A. M. and Bacillus tumescens Zopf.," 'Flora,' Bd. lxxxiv (Ergänzungsband), p. 185.

Meyer, A. (1899).—"Ueber Geisseln, Reservestoffe, Kerne und Sporenbildung der Bakterien," 'Flora,' Bd. lxxxvi, p. 428.

——— (1901).—"Ueber die Verzweigung der Bakterien," 'CB. Bakt.,' Abt. i, Bd. xxx, p. 49.

——— (1904).—"Orientirende Untersuchungen über Verbreitung, Morphologie und Chemie des Volutins," 'Bot. Ztg.,' Jahrg. lxii, p. 113.

——— (1908).—"Der Zellkern der Bakterien," 'Flora,' Bd. xcviii, p. 335).

Migula, W. (1894).—"Ueber den Zellinhalt von Bacillus oxalaticus Zopf.," 'Arb. bakteriol. Inst. Karlsruhe,' Bd. i.

——— (1897, 1900).—'System der Bakterien,' Jena (Fischer).

——— (1904).—'Der Bau der Bakterienzelle," Lafar's 'Handb. techn. Mykol.,' Bd. i, p. 48.

Mitrophanow, P. (1893).—"Études sur l'organisation des Bactéries," 'Monthly internat. Journ. Anat. Physiol.,' vol. x, p. 475.

Mühlens, P., and Hartmann, M. (1905).—"Ueber Bacillus fusiformis und Spirochæta dentium," 'Zeitschr. Hyg.,' Bd. lv, p. 81.

Mühlschlegel, A. (1900).—"Ueber die Bildung und den Bau der Bakteriensporen," 'CB. Bakt.,' Abt. ii, Bd. vi, p. 65.

Nakanishi, K. (1901).—"Ueber den Bau der Bakterien," 'CB. Bakt.,' Abt. i, Bd. xxx, p. 97.

Petschenko, B. (1908).—"Sur la structure et le cycle évolutif de Bacillopsis stylopygæ nov. gen. et nov. spec.," 'Bull. internat. Acad. Sci. Cracovie,' No. 4, p. 359.

Podwyssozki, W. W. (1893).—"Zur Morphologie der Choleravibrionen," 'CB. allg. Pathol.,' Bd. iv, p. 673.

Preisz, H. (1904).—"Studien über Morphologie und Biologie des Milzbrandbacillus," 'CB. Bakt.,' Abt. i, Bd. xxxv, p. 280.

Protopopoff (1891).—"Sur la question de la structure des Bactéries," 'Ann. Inst. Pasteur,' t. v, p. 332.

Raýman, B., and Kruis, K. (1904).—"Des noyaux des Bactéries," 'Bull. internat. Acad. Sci. Bohême.'

Rowland, S. (1899).—"Observations upon the structure of Bacteria," 'Trans. Jenner Inst. Prev. Med. London,' Series ii, p. 143.

Růžička, V. (1903).—"Ueber die biologische Bedeutung der färbbaren Körnchen des Bakterieninhaltes," 'Arch. Hyg.,' Bd. xlvi, p. 337.

——— (1908).—"Sporenbildung und andere biologische Vorgänge bei dem Bact. anthracis," 'Arch. Hyg.,' Bd. lxiv, p. 219.

——— (1909).—"Die Cytologie der sporenbildenden Bakterien und ihr Verhältnis zur Chromidiallehre," 'CB. Bakt.,' Abt. ii, Bd. xxiii, p. 289.

Schaudinn, F. (1902).—"Beiträge zur Kenntnis der Bakterien und verwandter Organismen: I. Bacillus bütschlii, n. sp.," 'Arch. Protistenk.,' Bd. i, p. 306.

——— (1903).—"II. Bacillus sporonema, n. sp.," 'Arch. Protistenk.,' Bd. ii, p. 421.

Schewiakoff, W. (1893).—"Ueber einen neuen bakterienähnlichen Organismus des Süsswassers," 'Verh. nat.-med. Ver. Heidelberg,' N.F. 5, p. 44.

Schottelius, M. (1888).—"Beobachtung kernartiger Körper im Innern von Spaltpilzen," 'CB. Bakt.,' Bd. iv, p. 705.

Schumburg, (1902).—"Die Beziehungen der Babes-Ernstschen Körnchen zu der Virulenz der Bakterien," 'CB. Bakt.,' Abt. i, Bd. xxxi, p. 694.

Sjöbring, N. (1892).—"Ueber Kerne und Theilungen bei den Bakterien," 'CB. Bakt.,' Bd. xi, p. 65.

Sulc, K. (1906).—"Kermincola kermesina n. g. n. sp. und physokermina n. sp., neue Mikroendosymbiotiker der Cocciden," 'SB. kgl. böhm. Ges. Wiss. Prag.'

——— (1910).—"Pseudovitellus und ähnliche Gewebe der Homopteren sind Wohnstatten symbiotischer Saccharomyceten," 'SB. kgl. böhm. Ges. Wiss. Prag.'

Swellengrebel, N. H. (1906).—"Zur Kenntnis der Zytologie von Bacillus maximus buccalis (Miller)," 'CB. Bakt.,' Abt. ii, Bd. xvi, p. 617.

——— (1907).—"Zur Kenntnis der Zytologie der Bakterien. I. Bacterium binucleatum," 'CB. Bakt.,' Abt. ii, Bd. xix, p. 193.

——— (1907A).—"Sur la cytologie comparée des Spirochètes et de Spirilles," 'Ann. Inst. Pasteur,' t. xxi, p. 448.

——— (1908).—"Erwiderung auf die Arbeit des Herrn Dr. Hölling: Spirillum giganteum und Spirochæta balbianii," 'CB. Bakt.,' Abt. ii, Bd. xlvi, p. 1.

——— (1909).—"Neuere Untersuchungen über die vergleichende Cytologie der Spirillen und Spirochaten," 'CB. Bakt.,' Abt. I, Bd. xlix, p. 529.

——— (1909A).—"Untersuchungen über die Zytologie einiger Fadenbakterien," 'Arch. Hyg.,' Bd. lxx, p. 380.

Trambusti, A., and Galeotti, G. (1892).—"Neuer Beitrag zum Studium der inneren Struktur der Bakterien," 'CB. Bakt.,' Bd. xi, p. 717.

Vay, F. (1910).—"Studien über die Strukturverhältnisse von Bakterien mit Hilfe von farbehaltigen Nährböden," 'CB. Bakt.,' Abt. i, Bd. lv, p. 193.

Vejdovský, F. (1900).—"Bemerkungen über den Bau und Entwicklung der Bakterien." 'CB. Bakt.,' Abt. ii, Bd. vi, p. 577.

——— (1904).—"Ueber den Kern der Bakterien und seine Teilung," 'CB. Bakt.,' Abt. ii, Bd. xi, p. 481.

——— (1906).—"Bemerkungen zum Aufsatze des Herrn Dr. K. Šulc über Kermincola kermesina, etc.," 'SB. kgl. böhm. Ges. Wiss. Prag.'

Wager, H. (1891).—"On a Nuclear Structure in the Bacteria," 'Ann. Bot.,' vol. v, p. 513.

——— (1895).—"Preliminary Note upon the Structure of Bacterial Cells," 'Ann. Bot.,' vol. ix, p. 659.

——— (1898).—"The Nucleus of the Yeast-Plant," 'Ann. Bot.,' vol. xii, p. 499.

Wagner, A. (1898).—"Coli- und Typhusbakterien sind einkernige Zellen. Ein Beitrag zur Histologie der Bakterien," 'CB. Bakt.,' Abt. i, Bd. xxiii, p. 433.

Wahrlich, W. (1890, 1891).—"Bakteriologische Studien, I and II" (Russian), in 'Scripta Botanica,' St. Petersburg. See Review in 'CB. Bakt.,' Bd. xi, 1892, p. 49.

Walker, E. W. A., and Murray, W. (1904).—"The Effect of Certain Dyes upon the Cultural Characters of the Bacillus typhosus and some other Micro-organisms," 'Brit. Med. Journ.,' vol. ii, p. 16.

West, G. S., and Griffiths, B. M. (1909).—"Hillhousia mirabilis, a Giant Sulphur Bacterium," 'Proc. Roy. Soc. London,' B. vol. lxxxi, p. 398.

Winogradsky, S. (1888).—'Beiträge zur Morphologie und Physiologie der Bacterien. Heft i, zur Morphologie und Physiologie der Schwefelbacterien,' Leipzig (Felix).

Zettnow, E. (1891).—"Ueber den Bau der Bacterien," 'CB. Bakt.,' Bd. x, p. 690.

——— (1897).—"Ueber den Bau der Grossen Spirillen," 'Zeitschr. Hyg.,' Bd. xxiv, p. 72.

——— (1899).—"Romanowski's Färbung bei Bakterien," 'Zeitschr. Hyg.,' Bd. xxx, p. 1.

——— (1900).—"Romanowski's Färbung bei Bakterien," 'CB. Bakt.,' Abt. i, Bd. xxvii, p. 803.

——— (1908).—"Ueber Swellengrebels Chromatinbänder in Spirillum volutans," 'CB. Bakt.,' Abt. i, Bd. xlvi, p. 193.

Ziemann, H. (1898).—"Eine Methode der Doppelfärbung bei Flagellaten, Pilzen, Spirillen und Bakterien, sowie bei einigen Amöben," 'CB. Bakt.,' Abt. i, Bd. xxiv, p. 945.

Zopf, W. (1885).—'Die Spaltpilze,' Breslau.

EXPLANATION OF PLATES 16–19,

Illustrating Mr. C. Clifford Dobell's "Contributions to the Cytology of the Bacteria."

[All the figures are drawn from fixed and stained organisms under a Zeiss 2 mm. apochromatic oil-immersion (aperture 1·40) with the aid of compensating oculars 6, 8, 12, and 18. The magnification of all figures is the same, and is approximately 2000 diameters. The figures are in no way diagrammatic. They are accurate representations of the actual appearances observed.]

PLATE 16.

Figs. 1–20 are from wet film preparations of the blood of *Saccobranchus fossilis*, fixed with osmic vapour followed by absolute alcohol, and stained with Giemsa's stain.

Figs. 1–10, 12 and 14.—*Bacillus saccobranchi* n. sp.

Fig. 1.—Short Bacillus, with nucleus in the form of a slightly bent and varicose filament.

Fig. 2.—Two Bacilli with nuclei in the form of twisted zig-zag or spiral filaments.

Fig. 3.—Bacillus with nucleus in the form of fragments of a zig-zag filament.

Fig. 4.—Long Bacillus containing a long varicose zig-zag or spiral nuclear filament. (Nucleus of *spirogyra* type.)

Fig. 5.—Large Bacillus in which the nucleus is in the form of granules and irregular short, curved, bent, and branched filaments. (Irregular type of nucleus.)

Fig. 6.—Large Bacillus with nucleus partly in the form of an irregular zig-zag or spiral filament and partly in the form of irregular branched masses—connected with one another.

Fig. 7.—Two short Bacilli with irregular nuclei.

Fig. 8.—Large, slightly curved Bacillus, with nucleus in the form of a broken varicose zig-zag or spiral filament.

Fig. 9.—Bacillus with nucleus of irregular type. A part of the nucleus shows a very distinct reticular arrangement.

Fig. 10.—Bacillus with nucleus of chromidial type.

Fig. 12.—Bacillus with nucleus in the form of a thick varicose filament.

Fig. 14.—Bacillus containing a large and almost fully formed spore. Residual chromatin is seen lying in the cytoplasm outside the spore.

Figs. 11, 13, 15–20.—Smaller Bacteria, found in company with B. saccobranchi.

Fig. 11.—Chain of three individuals with nuclei of spirogyra type.

Fig. 13.—Short, thick Bacillus with nucleus in the form of short, thick, irregular rodlet, pointed at one end.

Fig. 15.—Bacillus with nucleus in the form of a varicose spiral or zig-zag filament.

Fig. 16.—Bacillus with nucleus in irregular masses.

Fig. 17.—Two Bacilli with nuclei in the form of short, irregular rodlets.

Fig. 18.—A similar organism, with nucleus undergoing division.

Fig. 19.—Three very small Bacilli with nuclei of spirogyra type.

Fig. 20.—Group of five small Bacilli with spirogyra type of nucleus.

Figs. 21–23.—Bacilli of flexilis type, from large intestine of Mabuia carinata. (Osmic acid 1 per cent., drop method; Leishman's stain.)

Fig. 21.—Ordinary individual, with chromidial nucleus.

Fig. 22.—Similar individual. The chromidia are smaller and more numerous than in the preceding.

Fig. 23.—Spore-bearing (disporic) individual. The spore-coats are stained blue, and a certain amount of residual chromatin material is seen in the cytoplosm.

Figs. 24–29.—Sarcina from large intestine of Bufo melanostictus. (Osmic acid 1 per cent., drop method; Giemsa's stain.)

Fig. 24.—Organism in two-cell stage. Small spherical nuclei (red) in each cell. The upper cell contains a refractile granule (white).

Fig. 25.—Four-cell stage. In the upper left-hand cell the nucleus has divided into two. The three other cells each contain a single nucleus. In each cell a single refractile granule is present.

Fig. 26.—Three-cell stage. The nucleus in the right-hand cell has divided into two, but fission of the cytoplasm has not yet occurred. The upper and lower left-hand cells contain dividing nuclei, of characteristic dumb-bell form. A single large refractile granule is present in the right-hand cell; the left-hand cells each contain a single and smaller refractile granule.

Fig. 27.—Three-cell stage. The left-hand cells each contain a single nucleus and a single refractile granule. The right-hand cell shows a nucleus undergoing division.

Fig. 28.—Four-cell stage. Each cell contains a nucleus and a refractile granule.

Fig. 29.—Four-cell stage. The lower right-hand cell contains a single resting nucleus. The three other cells contain dividing nuclei. The upper left-hand cell contains two small refractile granules—the three others one.

Figs. 30–40.—Large Bacilli of spirogyra type from large intestine of Mabuia carinata. (1 per cent. osmic acid, drop method; Leishman's stain.)

Fig. 30.—Long Bacillus, with nucleus in the form of a spiral or zig-zag filament.

Fig. 31.—A similar form to the preceding, but with a longer and more twisted nucleus.

Fig. 32.—Similar form, showing two loops in the nuclear filament.

Fig. 33.—Similar organism just completing division into two.

Fig. 34.—Shorter individual, with typical spirogyra type of nucleus.

Fig. 35.—Similar form with nucleus in the form of a straighter, varicose filament.

Fig. 36.—Short Bacillus, with nucleus clearly seen to be composed of chromatin granules, aggregated to form a spiral or zig-zag filament.

Fig. 37.—Bacillus containing finely granular cytoplasm and six large nuclear granules. Possibly a degenerate or developmental form of the preceding organisms.

Fig. 38.—Bacillus with nucleus in the form of a broken spiral filament. Degenerate or developmental form?

Fig. 39.—Spore-bearing individual of spirogyra type.

Fig. 40.—Degeneration form.

Fig. 41.—Long, slender Bacillus from large intestine of Mabuia carinata. Nucleus of chromidial type. (1 per cent. osmic acid, drop method; Leishman's stain.)

Figs. 42–44.—Micrococci from large intestine of Mabuia carinata. (1 per cent. osmic acid, drop method; Leishman's stain.)

Fig. 42.—Diplococcus form—each cell with a single nucleus.

Fig. 43.—Coccus with nucleus in the form of a short zig-zag filament.

Fig. 44.—Typical Micrococcus, with single nuclear granule.

PLATE 17.

Figs. 45–60.—Various Bacteria from large intestine of Lacerta muralis. (1 per cent. osmic acid, drop method; Giemsa's stain.)

Fig. 45.—Group of five Micrococci of different sizes. The nucleus is very obvious in each cell.

Figs. 46–49.—Four successive stages in the division of a Micrococcus similar to those seen in the preceding figure. Note the characteristic dumb-bell figure assumed by the nucleus during division. (Compare with figs. 24–29, Plate 16.)

Fig. 50.—Three coccobacillar forms. The nucleus is in the form of a filament, bent in a more or less spiral or zig-zag manner.

Fig. 51.—Group of short Bacilli, with nuclei of characteristic spirogyra form.

Fig. 52.—Chain of Cocci in which division is taking place. Note the zig-zag or spiral form assumed by some of the dumb-bell figures of the dividing nuclei. (This figure is drawn on a very slightly larger scale than the others.)

Fig. 53.—Small Bacillus with nucleus in the form of a short rod.

Fig. 54.—Similar organism to the preceding, in the act of dividing into two. The nuclear rod is completely divided into two parts.

Figs. 55–60.—Large Bacilli of spirogyra type.

Figs. 55–57.—Three individuals, showing three different arrangements of the nuclear filament.

Fig. 58.—Spore-bearing individual of same species. (A single terminal spore is formed—as in B. spirogyra.)

Fig. 59.—Dividing individual. The two halves of the nuclear filament are still joined by a very slender chromatin thread.

Fig. 60.—Another dividing individual. The nucleus—which is very much contorted (cf. fig. 59)—has already separated into two parts.

Fig. 61.—Three short Bacilli, with nuclei of spirogyra type, from large intestine of Bufo melanostictus. (1 per cent. osmic acid, drop method; Giemsa's stain.)

Fig. 62.—Long, curved Bacillus, with irregular varicose nuclear filament. Large intestine of Bufo melanostictus. (1 per cent. osmic acid, drop method; Giemsa's stain.)

Fig. 63.—Group of Bacilli from large intestine of Lacerta muralis. The nucleus is in the form of an irregular knotted rodlet. The lowest organism is undergoing division—the nucleus being already divided into two. (1 per cent. osmic acid, drop method; Giemsa's stain.)

Figs. 64, 65.—Slender Bacilli from large intestine of Lacerta muralis. Nucleus in the form of chromidia. (1 per cent. osmic acid, drop method; Giemsa's stain.)

Figs. 66, 67.—"Fusiform Bacteria" from large intestine of Triton vulgaris. The upper individual of the pair shown in fig. 66 is dividing. Note the nuclei—in the form of double granules. Fig. 67 is

a double form, with one nucleus (upper) appearing as a solid mass of chromatin, the other (lower) as a vesicular structure with a large karyosome. (40 per cent. formol, drop method, absolute alcohol; Giemsa's stain.)

Fig. 68.—"Fusiform Bacterium" (double form) from large intestine of Lacerta muralis. Each individual possesses a small spherical nucleus. (Dry film, absolute alcohol; Giemsa's stain.)

Figs. 69–78.—Bacilli from large intestine of Mabuia carinata. (Osmic acid 1 per cent., drop method; Leishman's stain).

Fig. 69.—Long slender Bacillus with nucleus of spirogyra type.

Figs. 70–73.—Smaller Bacilli of spirogyra type. Diverse forms and sizes.

Fig. 74.—Very small Bacillus with thick nuclear filament of spirogyra type.

Fig. 75.—A Bacillus, similar to that shown in fig. 72, undergoing fission.

Fig 76.—Slender Bacillus with nucleus of chromidial type.

Fig. 77.—Slender Bacillus with large central nuclear mass (possibly a plasmolysed form?).

Fig. 78.—Bacillus with nucleus in the form of a short, irregular, and slightly bent rod-like filament.

Figs. 79–82.—Bacilli of modified flexilis form from large intestine of Triton vulgaris. (40 per cent. formol, absolute alcohol; Giemsa's stain.)

Fig. 79.—Bacillus of flexilis form, with chromidial nucleus.

Fig. 80.—Individual with finely granular, darkly staining cytoplasm, and large nucleus-like masses of chromatin, eight in number.

Fig. 81.—Long, sporulating individual, bearing a large chromatin spore-rudiment at each end. (The organism is normally disporic, like B. flexilis.)

Fig. 82.—Long individual similar to that shown in fig. 80. The cytoplasm is alveolar, and the chromatin in the form of large nucleus-like masses.

Figs. 83, 84.—Long and short individuals respectively of Bacillus of flexilis type from large intestine of Lacerta muralis. Nuclei of chromidial form. (Osmic vapour [wet film], absolute alcohol; Giemsa's stain.)

Figs. 85–90.—Bacilli of modified flexilis form from large intestine of Lacerta muralis. (Dry film, absolute alcohol; Giemsa's stain.)

Fig. 85.—Long individual, containing three large nucleus-like masses of chromatin.

Fig. 86.—Short individual, with curious arrangement of the chromatin.

Fig. 87.—Large individual, somewhat similar to the preceding.

Fig. 88.—Short individual with a single, centrally placed, nucleus-like body.

Fig. 89.—Long, sinuous individual, with chromidial nucleus of characteristic flexilis type. Many of the chromidia are conspicuous by their large size.

Fig. 90.—Long, straight Bacillus, with chromatin mainly in two large masses. Possibly a plasmolysed or degenerate form.

PLATE 18.

[All the figures, unless otherwise stated, are drawn from wet film preparations fixed with Schaudinn's sublimate-alcohol, and stained with Heidenhain's iron-hæmatoxylin.]

Figs. 91–95.—Bacilli of spirogyra form from large intestine of Lacerta muralis. Various forms of nuclear filament are shown. The organism depicted in fig. 92 is dividing.

Figs. 96–108.—Large Spirilla from large intestine of Lacerta muralis.

Fig. 96.—Short individual, showing large cytoplasmic alveoli and nucleus in the form of a short rod-like filament at one end of the cell.

Figs. 97, 98.—Dividing forms. Note nuclear filaments.

Fig. 99.—Short individual, with nucleus in the form of a short and somewhat zig-zag or spiral filament.

Fig. 100.—Short individual with long, varicose nuclear filament.

Fig. 101.—Longer individual, with long spiral or zig-zag nuclear filament.

Fig. 102.—Very long individual, with long nuclear filament similar to that of the preceding.

Figs. 103–105.—Shortest individuals (Vibrio form) with nuclear filaments.

Fig. 106.—A form similar to fig. 99, but with a longer nuclear filament.

Fig. 107.—Longer organism, with short, centrally placed nuclear filament.

Fig. 108.—Long individual, in which the nuclear filament has divided into two preparatory to cell division.

Fig. 109.—Group of five small Bacilli with darkly staining nucleus-like bodies—similar to those shown in fig. 53 (Pl. 17). (These "nuclei" are possibly spore-rudiments.) From large intestine of Lacerta muralis.

Figs. 110–112.—Large Spirilla from the hind gut of Stylopyga orientalis. The cytoplasm has an alveolar structure, and the nucleus is of the chromidial type.

Figs. 113 and 114.—"Fusiform Bacteria" from the large intestine of Lacerta muralis. Each cell shows a single spherical nucleus.

Fig. 115.—"Fusiform Bacterium," of double form, from large intestine of Bufo vulgaris. (Fixation: corrosive sublimate and acetic acid.)

Fig. 116.—"Fusiform Bacterium," of double form, from large intestine of Stylopyga orientalis. The lower nucleus in dividing.

Figs. 117 and 118.—Bacillus spirogyra from large intestine of Bufo vulgaris. Note the nuclear filaments. (Fixation: corrosive sublimate and acetic acid.)

Figs. 119 and 120.—Bacillus flexilis from large intestine of Bufo vulgaris. Note the alveolar structure of the cytoplasm (rather indistinct) and the nucleus in the form of chromidia. The organism shown in fig. 120 is undergoing division.

Figs. 121–132.—Small Spirilla from the hind gut of Stylopyga orientalis.

Fig. 121.—Small Vibrio form with terminal nucleus.

Figs. 122–124.—Small individuals with centrally situate nuclei.

Figs. 125 and 126.—Longer individuals with dividing nuclei. Note the characteristic dumb-bell figure which the nucleus assumes. (Compare with Micrococci and Sarcina.)

Fig. 127.—Individual in which nucleus has divided into two, though fission of the cytoplasm has not yet occurred.

Figs. 128 and 129.—Dividing organisms.

Fig. 130.—Long individual with centrally placed, undivided nucleus.

Fig. 131.—Small Vibrio form. Central nucleus.

Fig. 132.—Smallest Vibrio form. Central nucleus in the form of a minute chromatin granule.

Figs. 133 and 134.—Bacilli of flexilis type from large intestine of Lacerta muralis. Chromidial nuclei. Fig. 134 shows a dividing individual. Same forms as those shown in figs. 83, 84 (Plate 17).

Figs. 135 and 136.—Bacterium-like organism from large intestine of Boa constrictor. (Wet film, absolute alcohol; Delafield's hæmatoxylin.)

Fig. 135.—Bacterioid forms—a chain of four.

Fig. 136.—Zymoid forms. A free single individual and another, which has formed a bud.

PLATE 19.

[All figures are of the nucleated Bacterium-like organism (or its developmental forms) found in the large intestine of Boa constrictor. (Dry film preparations: fixed absolute alcohol, stained Giemsa.)]

Figs. 137–141, 144.—Bacterioid forms.

Fig. 142.—Form intermediate between bacterioid and zymoid form.

Figs. 143 and 145.—Zymoid forms.

Figs. 146, 147, 149, 150.—Zymoid forms, producing outgrowths. In fig. 150 the outgrowth has divided off as a more or less bacterioid cell.

Fig. 148.—Four bacterioid forms in a chain—the two middle individuals producing outgrowths.

Fig. 151.—A chain composed of both zymoid and bacterioid individuals.